

PB - 240 173

STUDY OF THE ECOLOGY OF PESTICIDES

W. B. DEICHMANN

Miami University

Prepared for:

National Environmental Research Center

October 1974

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE

TECHNICAL REPORT DATA		
1. REPORT NO. EPA-650/1-74-012	2.	3. PB 240 173
4. TITLE AND SUBTITLE Study of the Ecology of Pesticides		5. REPORT DATE October 1974
7. AUTHOR(S) W. E. Deichmann		6. PERFORMING ORGANIZATION CODE
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Miami, School of Medicine Research and Teaching Center of Toxicology P.O. Box 8216 Coral Gables, Florida 33124		8. PERFORMING ORGANIZATION REPORT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Protection Agency National Environmental Research Center Research Triangle Park, N.C. 27711		10. PROGRAM ELEMENT NO. IE1078
		11. CONTRACT/GRANT NO. R801470
15. SUPPLEMENTARY NOTES		13. TYPE OF REPORT AND PERIOD COVERED Final Report 1973-1974
16. ABSTRACT Mean survival of the groups of male mice fed dieldrin 0, 3, and 10 ppm for a lifetime, ranged from 12.0 to 13.3 months. Mean survival of the females ranged from 18.6 to 21.8 months. While there was an increase in liver weights, and an increase in the ratio of liver-to-body weight of the male and female mice fed dieldrin 10 ppm, the differences were not statistically significant. The number of male and female mice with benign tumors was essentially the same in the three groups: a total of 26 of 163 control mice, 29 of 159 mice fed dieldrin 3 ppm, and 27 of 161 mice fed dieldrin 10 ppm. When compared with the controls, the number of male and female mice showing malignant tumors decreased as the feeding levels of dieldrin increased: from 40 control mice to 37 mice fed dieldrin 3 ppm, and to 16 mice fed dieldrin 10 ppm. Review of selected liver slides by several pathologists demonstrated a difference in their diagnosis of the lesions as being either non-neoplastic or neoplastic. Difficulties in reaching a unanimous decision have been recognized for some time and became the subject of discussion at the working conference on liver cancer in the summer of 1969, organized by WHO International Agency for Research on Cancer. In the aldrin-dieldrin-endrin feeding study (Deichmann et al., Ind. Med. Surg. 39:426-434, 1970), the number of animals dying with tumors was related to the total number of rats with which the feeding experiment was initiated. Recalculation of the data, but based on the number of male and female rats that were actually at tumor risk, confirmed the original conclusions that there were no statistically significant differences in the tumor incidence between the control and experimental rats. A second rat-feeding experiment with aldrin was conducted with two strains of weanling female rats: Osborne-Mendel and Sprague-Dawley (50 rats per dose level). The reasons for the use of female rats were that in the 1970 aldrin-dieldrin feeding experiment (1) tumors were noted most frequently in females, i.e., mammary tumors; (2) signs of acute toxicity, a reduced life span, and a marked increase in the ratio of liver-to-body weight were noted primarily in the female rats. Two strains were used, since susceptibility to tumors varies. Based on (1) the incidence of all tumors found at necropsy in the female Osborne-Mendel and Sprague-Dawley rats fed aldrin with the diet at 0, 20, and 50 ppm, and on (2) an age-adjusted risk analysis carried out on the number of rats that developed mammary tumors, it is concluded that the lifetime feeding of aldrin 20 and 50 ppm neither increased nor decreased the incidence of tumors in these two strains of female rats above that noted in the respective control groups. It will require completion of the microscopic studies to determine whether the feeding of aldrin altered the distribution of benign and malignant tumors. In the third study, Swiss-Webster mice were fed aldrin 5 and 10 ppm, and DDT 100 ppm, for seven generations. As the generations progressed, particularly from F3 on, conception was frequently delayed from three to four weeks. No other signs of toxicity were noted. When sacrificed at age 260 $\pm$ 15 days, total body lipids in the F1 generations of all groups showed statistically significant increases when compared with the respective parent (P) generations. In subsequent generations, total body lipids showed no specific trend with the exception of mice fed aldrin 10 ppm, in which total body lipids remained elevated from generations F1 to F6. In all generations, the pesticide concentrations in abdominal fat were essentially the same as the pesticide concentrations in total body lipids, except in mice of the P generation fed DDT 100 ppm, in which the concentrations of total DDT in abdominal fat were approximately twice the concentrations in the lipids of the total body. All experimental mice showed a significant increase in both concentration and total retention of dieldrin or total DDT in the total carcass of the second generation (F1) when compared with the P generation. Feeding of the pesticide-free diet to all experimental mice of the F4 generations resulted in the complete excretion of the pesticides absorbed by these animals while in utero and with their mothers' milk. Resumption of the feeding of the experimental diets by generations F5 and F6 resulted in markedly elevated body pesticide retention and in markedly increased concentrations of pesticides (dieldrin or DDT plus metabolites) in the total body lipids, and to some further increases in concentration or retention in the F6 generations.		14. SPONSORING AGENCY CODE
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Carcinogenicity Mice Aldrin Reproduction Retention Dieldrin	Liver Tumors DDT Swiss-Webster Seven Generation Rats	
19. DISTRIBUTION STATEMENT Unlimited		19. SECURITY CLASS (This Report) Unclassified
		20. SECURITY CLASS (This page) Unclassified
		21. NO. OF PAGES 56
		22. PRICE \$4.25

STUDY OF THE ECOLOGY OF PESTICIDES

by

W.B. Deichmann
University of Miami
School of Medicine
P.O. Box 8216
Coral Gables, Florida 33124

Grant No. R801470
ROAP No. 21AFM, Task No. 54
Program Element No. 1E1078

EPA Project Officer: Dr. William F. Durham

Pesticides and Toxic Substances Effects Laboratory
National Environmental Research Center
Research Triangle Park, N.C. 27711

Prepared for

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

October 1974

ia

This report has been reviewed by the Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

TABLE OF CONTENTS

Part 1	Carcinogenicity: Effect in Mice Following Prolonged Feeding of Dieldrin	1
Part 2	Effects of Feeding Aldrin and Dieldrin on the Tumor Incidence in Albino Rats	17
Part 3	Mouse Reproduction Study: Seven-Generation Study: Retention of Dieldrin and DDT in the Tissues of Mice Fed Aldrin and DDT	37

Part 1

CARCINOGENICITY: EFFECT IN MICE FOLLOWING PROLONGED FEEDING OF DIELDRIN

INTRODUCTION

The members of the Aldrin-Dieldrin Advisory Committee* (1963-1965) found available data on rat and mouse studies "...inconclusive as far as carcinogenesis is concerned." Believing that the use of aldrin and dieldrin might continue indefinitely for certain purposes, they suggested that additional studies be initiated. The mouse studies reported here were conducted for the primary purpose of providing information on the tumorigenicity or carcinogenicity of dieldrin.

EXPERIMENTAL

A total of 684 weanling Swiss-Webster mice were divided into three groups. Group 1 (125 males and 100 females) were fed the control diet of ground Purina Laboratory Chow; Group 2 (129 males and 100 females) were fed the laboratory chow supplemented with 3 ppm of dieldrin; and Group 3 (130 males and 100 females) were fed the chow diet supplemented with 10 ppm of dieldrin. During the first two months, the mice were fed half of these concentrations of dieldrin in the diet. The animals were housed 10 to a cage and maintained in a room air-conditioned by a single unit (thus avoiding exposure to chemicals that might find their way into a general laboratory airconditioning system).

All animals were observed daily; those that were ill (particularly males, because of fighting) were sacrificed by decapitation and subjected to necropsy. All livers, all tumors, and all organs showing gross anatomical changes were preserved for histological study. The weights of the livers were recorded.

RESULTS AND SUMMARY

Table 1 presents the ratio of liver-to-body weight of the male and female mice fed 0, 3, and 10 ppm of dieldrin. Analysis of the data by the student "t" test indicates that the differences noted, particularly the increase in the liver-to-body weight ratio in the animals fed dieldrin 10 ppm, are statistically not significant ($p > 0.05$).

* Appointed in 1963 at the recommendation of the Secretary of HEW, Committee comprised of: Hans Popper, M.D., Chairman; Alfred M. Boyce, Ph.D.; Wm. B. Deichmann, Ph.D.; Harold C. Hodge, Ph.D.; Henry F. Kraybill, Ph.D.; James A. Miller, M.D.; and Albert Tannenbaum, M.D. Subcommittee on Carcinogenesis: Drs. Miller, Popper, and Tannenbaum.

Table 1. RATIO OF LIVER TO BODY WEIGHT OF MICE FED DIELDRIN

Sex	Body Weight (g)	Liver Weight (g)	Ratio of Liver-to-Body Weight
<u>C o n t r o l s</u>			
M	25.2±8.5	3.1±2.0	0.088
F	32.0±7.6	2.8±2.1	0.088
<u>D i e l d r i n 3 ppm</u>			
M	30.1±7.3	2.8±1.4	0.093
F	31.2±6.8	2.6±1.3	0.083
<u>D i e l d r i n 10 ppm</u>			
M	31.1±5.8	3.4±1.2	0.109
F	30.9±6.6	3.9±1.7	0.125

The relation of tumor deaths (mice dying with one or more tumors) to total fatalities among the mice fed 0, 3, and 10 ppm of dieldrin, is presented in Table 2. While the mean periods of survival of the control and experimental male mice showed no significant differences (Table 3), the incidence of deaths with tumors decreased from 23 per cent in the controls to 14 and 11 per cent, respectively, in the groups fed 3 and 10 ppm of dieldrin. The relatively short life span of the male mice was due to fighting and subsequent infections.

The mean life span of the female control mice was 18.6 months. While not statistically significant, the mean life span of the experimental female mice was more prolonged than that of the controls; in the females fed dieldrin 3 ppm, it was 20.7 months, and in the dieldrin 10 ppm group it was 21.8 months. The incidence of deaths with tumors as related to the total number of deaths was 63 per cent of the female control group, slightly higher, namely 70 per cent, for the group of mice fed dieldrin 3 ppm, and considerably lower -- 47 per cent -- for the female mice fed dieldrin 10 ppm.

Considering the number of mice per group examined histologically, which ranged, for the males, from 81 to 93, and for the females from 70 to 78, and considering that the life spans of the experimental male and female mice compared well with those of their respective control groups, it must be concluded that the reduction in the number of tumor deaths -- in all groups of experimental mice, except in the females fed dieldrin 3 ppm -- is rather noteworthy.

Table 3 presents the survival periods and the incidence of pathological lesions (except in the liver) diagnosed microscopically. For each of the three feeding groups (0, 3, and 10 ppm), the incidence of micropathological changes was as follows:

Kidney:

Males - benign lesions, 0, 0, and 1 (adenoma), respectively;

Females - benign lesions, 0, 0, and 0, respectively.

Mammary and Subcutaneous:

Males - primary malignant lesions, 1, 1, and 0, respectively;

Females - primary malignant lesions, 11, 15, and 3, respectively (carcinoma and adenocarcinoma).

Males - benign lesions, 0, 0, and 1 (sebaceous adenoma);

Females - benign lesions, 0, 0, and 1 (hemangioma).

Uterus:

Females - adenomatous hyperplasia, 12, 15, and 13.

- other benign lesions, 2, 1, and 0 (control: leiomyoma and hemangioma; 3 ppm: fibroma).

Table 2
Relation of Tumor Deaths to Total Fatalities in Mice Fed Dieldrin in the Diet
(As Confirmed by Micropathological Examination)

Months of Feeding Experiment																																Per Cent Tumor Deaths	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
Males																																	
Control Diet																																	
Monthly Tumor Deaths						2	1	1	3	2	3		2	1																			
Monthly Fatalities	2	2	5	2	7	4	17	10	8				5	2	3	2	1	4	4	4	3	2	1									21	
Dieldrin 3 ppm																																	
Monthly Tumor Deaths						1		6																									
Monthly Fatalities	1				1	5	5	7	6	12	16		5	13	4	2																11	
Dieldrin 10 ppm																																	
Monthly Tumor Deaths						2	1		2	1			1																				
Monthly Fatalities	1	2	1		4	4	7	5	4	5	14		6	9	5	1	6	3	3	1	6											10	
Dieldrin 10 ppm																																	
Monthly Tumor Deaths																																	
Monthly Fatalities	1	2	1																														91
Females																																	
Control Diet																																	
Monthly Tumor Deaths						1	1		2	1	3		1	2	3	2	3	4	5	1													
Monthly Fatalities	1	1				1	1		1	2	3	2	3	1	3	6	2	5	7	5	2	1	7	9	4	2	1		1	3		45	
Dieldrin 3 ppm																																	
Monthly Tumor Deaths						1	1	2																									
Monthly Fatalities	2	2	2			3	1						1	1	3	3	3	6	8	2	2	7	5	4	5	5	4	1	2		4	55	
Dieldrin 10 ppm																																	
Monthly Tumor Deaths									1																								
Monthly Fatalities	1																																
Dieldrin 10 ppm																																	
Monthly Tumor Deaths																																	
Monthly Fatalities	1																																

Table 3. MACROPHAGE-CELL REACTIONS OF TUMORS (EXCEPT LIVER TUMORS) IN SWISS-WEBSTER MICE FED DIET FOR A LIFETIME

No. of Survival Mice (months)	No. of Mice Examined (months)	No. of Mice Bearing Tumors Significantly	Kidney	Lung	Lymphoid Tissues	Subcutaneous Tissues	Uterus of Prostate	Gonads	Gastrointestinal Tract	Other
C o n t r o l s										
M	12-783.7	125/115*	93	11	10	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma
F	18-894.2	100/76	70	13	30	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma
D i e t i n										
M	12-083.9	139/101	81	8	3	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma
F	20-794.8	100/91	78	21	36	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma
D i e t i n 10 ppm										
M	13-344.5	130/110	91	6	6	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma
F	21-894.1	100/81	70	21	12	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adenoma Leukemic infiltration Basophilic	Adrenal, amyloidosis Skin, lipoma Adrenal, cortical adenoma

* Number of mice started over number of mice autopsied.

Ovaries:

- Females - primary malignant lesion, 0, 0, and 1 (adenocarcinoma);
- benign lesions, 0, 0, and 5 (cystadenoma, hemangioma);
- cystic changes, 4, 13, and 13.

Lymphoid Tissue:

- Males - malignant lesions, 5, 1, and 1 (malignant lymphoma);
Females - malignant lesions, 6, 12, and 4 (malignant lymphoma).

Gastroenteric Tract:

- Males - primary malignant lesions, 0, 0, and 0;
Females - primary malignant lesions, 2, 1, and 0 (controls: adenocarcinoma and sarcoma; 3 ppm: carcinoma)

Males - polypoid hyperplasia, 6, 4, and 1;
Females - polypoid hyperplasia, 5, 7, and 2.

Lung:

- Males - primary malignant lesions, 4, 0, and 1 (adenocarcinoma);
Females - primary malignant lesions, 8, 5, and 2 (adenocarcinoma).

Males - benign lesions, 2, 2, and 3 (adenomas);
Females - benign lesions, 6, 3, and 3 (adenomas).

Pancreas:

- Males - benign lesions, 0, 0, and 0;
Females - benign lesions, 0, 1, and 0 (islet cell hyperplasia).

Skin:

- Males - primary malignant lesions, 0, 0, and 0;
Females - primary malignant lesions, 5, 1, and 0 (carcinoma).

Skin (continued)

Males - benign lesions, 1, 0, and 0 (lipoma);

Females - benign lesions, 2, 0, and 0 (papilloma).

Tables 2 and 3 and the above histopathological summary indicate that:

- a) The mean survival periods of the control and experimental male mice (12.7, 12.0, and 13.3 months for the animals fed 0, 3, and 10 ppm of dieldrin) were considerably below those of the female mice (18.6, 20.7, and 21.8 months, respectively, for the three feeding groups).
- b) Undoubtedly because of the reduced life span of the male mice, the total number of deaths with benign or malignant tumors was considerably below the total number of tumor deaths noted in the female mice. For the 0, 3, and 10 ppm feeding groups, the percentage of tumor deaths among the males was 23, 14, and 11; for the females it was 63, 70, and 47 per cent.
- c) While benign and malignant tumors were found in some mice of all groups, clear-cut differences between experimental and control mice were noted only as follows: among the female mice fed dieldrin 10 ppm there was one mouse showing an adenocarcinoma and five mice with cystadenoma and hemangioma of the ovaries.* Cystic changes in the ovaries were noted in 4, 13, and 13 mice fed 0, 3, and 10 ppm of dieldrin, respectively. Malignant lymphoma and the associated leukemic infiltrations of many tissues (lung, spleen, pancreas, kidney, liver, etc.) were noted particularly in the females fed dieldrin 3 ppm. These changes were noted in 6, 12, and 4 female mice of the groups fed 0, 3, and 10 ppm of dieldrin, respectively. The female mice fed dieldrin 3 or 10 ppm, when compared with the female controls, showed a lower incidence of benign neoplasia of the uterus, benign and malignant neoplasia of the lung, polypoid hyperplasia and malignant neoplasia of the gastroenteric tract, and benign and malignant neoplasia of the skin. Most of the decreases were dose-related. The male mice fed dieldrin 3 or 10 ppm, when compared with the male control group, showed a lower incidence of malignant neoplasia of the lung and lymphoreticular system and polypoid hyperplasia of the gastroenteric tract.

LIVER: Since organochlorine pesticides are known to produce liver damage in certain strains of mice when fed in toxic concentrations for a lifetime, the micropathological diagnoses of liver lesions are presented in detail in Table 4.

Of the 15 types of non-neoplastic lesions of the liver listed in the table, marked differences from group to group were noted only in regard to dilatation of the sinusoids with associated protein precipitation and hepatic cellular swelling and atrophic changes.

* There were no neoplastic lesions in the controls and those fed 3 ppm.

Table 4. MICROPATHOLOGICAL DIAGNOSIS OF LIVER LESIONS
IN SWISS-WEBSTER MICE FED DIELDRIN FOR A LIFETIME

Sex	Number of Mice Examined Histo- logically	NON-NEOPLASTIC LESIONS												NEOPLASTIC LESIONS							
		Pigmented Kupfer Cells	Inflammatory Cell Infiltration	Congestion	Necrosis	Abscess	Dilated Sinusoids	Protein Precipitate in Sinusoids	Amyloid	Hyalin	Fibrosis	Fatty Change	Cellular Swelling	Giant Nuclei	Atrophic Changes	Hyperplasia	Duct Adenoma	Hemangioma	Primary Tumor	Leukemic Infiltration	Secondary Tumor
- CONTROL -																					
M	93	0	44	13	10	12	17	7	0	1	5	13	2	5	13	0	0	1	0	6	0
F	70	2	16	4	5	4	7	5	0	0	0	9	2	3	1	0	0	0	1*	4	0
- DIELDRIN 3 PPM -																					
M	81	2	39	8	3	9	28	21	1	0	1	1	10	3	18	0	0	0	0	1	0
F	78	4	36	10	3	10	8	8	0	0	10	11	3	4	20	0	0	0	0	9	0
- DIELDRIN 10 PPM -																					
M	91	1	42	18	14	10	7	6	1	0	0	3	11	5	44	1	0	0	0	2	0
F	70	0	24	4	3	3	5	1	0	0	1	7	18	1	20	0	1	0	0	1	0

* Hepatoma with fibrous component (D-2928)

All groups of male and female mice, but particularly the males and especially those fed dieldrin 3 ppm and the control males, showed dilatation of the sinusoids and protein precipitate in the sinusoids (a condition resembling amyloidosis). In the male mice fed 0, 3, and 10 ppm of dieldrin, dilatation of the sinusoids was noted in 17 of 93 control mice, in 28 of 81 mice fed 3 ppm, and in 7 of 91 mice fed 10 ppm. Protein precipitate was noted in 7, 21, and 6 male mice fed dieldrin 0, 3, and 10 ppm, respectively. The incidence of these changes in the control and experimental female mice was not able.

There was a dose-related increase in cellular swelling and in the incidence and degree of severity of atrophic hepatic changes -- in 2 mice (cellular swelling) and 13 mice (atrophic changes) of 93 control males, in 10 mice and 18 mice, respectively, of 81 males fed dieldrin 3 ppm, and in 11 mice and 44 mice of 91 males fed dieldrin 10 ppm. In the female mice, the changes were noted in 2 mice (cellular swelling) and one mouse (atrophic changes) of the 70 control mice, in 3 mice and 20 mice, respectively, of 78 mice fed dieldrin 3 ppm, and in 18 mice and 20 mice of 70 mice fed dieldrin 10 ppm.

Neoplastic lesions of the liver were noted only in a few animals. Among the male control mice there was one animal with hemangioma and six mice with leukemic infiltration. One of the male mice fed dieldrin 3 ppm and two of those fed dieldrin 10 ppm developed leukemic infiltration. The only primary malignant hepatocellular lesion in the entire experiment was noted in a control female mouse (diagnosed as a hepatoma with fibrous component, D-2928).

Four female control mice and nine females fed dieldrin 3 ppm showed leukemic infiltration. Of the females fed dieldrin 10 ppm, one animal exhibited leukemic infiltration and one showed duct adenomas.

CONCLUSIONS

Dieldrin was fed as a dietary supplement to 259 male and 200 female weanling Swiss-Webster mice. The control group included 125 male and 100 female weanling Swiss-Webster mice. No signs of intoxication were observed.

Survival: The mean survival of the three groups of male mice fed dieldrin 0, 3, and 10 ppm, ranged from 12.0 to 13.3 months. (The cause of early deaths was excessive fighting.) The mean survival of the three groups of female mice ranged from 18.6 to 21.8 months. While the experimental male and female mice -- except the males fed dieldrin 3 ppm -- survived the control mice, the differences were statistically not significant.

Body and Liver Weights: All animals gained at a normal rate. At death or sacrifice, the body weights of the control and experimental male and female mice did not differ significantly. While there was an increase in liver weights, and an increase in the ratio of liver-to-body weight of the male and female mice fed dieldrin 10 ppm, the differences were not statistically significant.

Micropathology: A total of 163 control mice, 159 mice fed dieldrin 3 ppm, and 161 mice fed dieldrin 10 ppm, were examined histologically. The number of male and female mice showing benign tumors was essentially the same in the three groups -- a total of 26, 29, and 27 in the groups fed dieldrin 0, 3, and 10 ppm, respectively.

When compared with the controls, the number of male and female mice showing malignant tumors decreased as the feeding level of dieldrin increased from 40 control mice with malignant tumors to 37 mice fed dieldrin 3 ppm, and to 16 mice fed dieldrin 10 ppm.

Male Mice: The feeding of dieldrin in concentrations of 0, 3, and 10 ppm to male mice resulted in a dose-related increased incidence and degree of severity in cellular swelling and atrophic changes in the liver, but in a dose-related decrease in the incidence of leukemic infiltration. There was also a dose-related decrease in malignant lesions of the lymphoreticular system and in polypoid hyperplasia of the gastroenteric tract. Finally, there was a decrease -- not dose-related -- in the incidence of neoplastic lesions of the lung.

Female Mice: The feeding of dieldrin in concentrations of 0, 3, and 10 ppm to female mice resulted in a marked increase in the incidence and severity of atrophic changes of the liver. But there was an essentially dose-related decrease in benign neoplasia of the uterus, skin, and lungs. Malignant neoplasia of the gastroenteric tract and skin in the animals fed 0, 3, and 10 ppm, showed a dose-related decrease.

Compared to the controls, the female mice fed dieldrin 3 ppm showed an increase in polypoid hyperplasia of the gastroenteric tract, but of the three groups, the females fed dieldrin 10 ppm showed the lowest incidence of these lesions. This decrease occurred in spite of the fact that the females fed dieldrin 10 ppm demonstrated the longest mean life span.

In the females fed 10 ppm, the ovaries and the mammary and subcutaneous tissues showed an increased incidence of neoplastic lesions. These lesions were absent in both the control females and those fed dieldrin 3 ppm.

The primary contributors to this investigation include:

William E. MacDonald, Ph.D., Co-Principal Investigator
William B. Deichmann, Ph.D., M.D. (Hon.), Principal Investigator
Edward B. Blum, M.D., Pathologist
Michael Bevilacqua, M.D., Pathologist
W. A. D. Anderson, M.D., Pathologist
Joseph Freal, M.S., Chemist
Mary D. Balkus, Technician

M. D. Reuber, M.D., contributed to the pathological review
described in the Appendix.

Wm. B. Deichmann

APPENDIX
Project No. 1

REVIEW BY SEVERAL PATHOLOGISTS OF SELECTED
LIVER SLIDES OF MICE FED DIELDRIN 0, 3, AND 10 PPM

Following the preliminary examination of all liver sections in our laboratory by W. E. MacDonald, Ph.D., 25 slides were selected for review by several pathologists. These included eight slides from control mice, seven slides from mice fed dieldrin 3 ppm, and 10 slides from mice fed dieldrin 10 ppm (Table 5). All sections were cut from tissues fixed in buffered formalin solution and stained with hematoxylin-eosin.

The pathologists* who diagnosed the hepatic lesions included our laboratory consultants, E. Blum, M.D. and M. Bevilacqua, M.D., W.A.D. Anderson, M.D., and M.D. Reuber, M.D.

The slides were selected for review with the intent of presenting a range of micropathological changes from essentially normal -to- various degrees of inflammatory and degenerative changes -to- nodulation and neoplastic lesions.

The information presented in Table 5 demonstrates that the pathologists disagreed markedly in their diagnoses of the lesions as being either non-neoplastic or neoplastic. This is a serious problem, the existence of which has been recognized by pathologists and toxicologists for some time, since it prevents an investigator who is truly seeking the facts from concluding that a chemical is either a hepatic carcinogen or a noncarcinogenic compound.

This dilemma was discussed at the working conference on liver cancer in the summer of 1969, organized by the WHO International Agency for Research on Cancer**. This group recommended that the term "hepatoma" be abandoned because of the difference in meaning to clinical and experimental pathologists. It was also recognized at this conference that "restorative hyperplasia" is a better pathological term than "regeneration," and "nodular hyperplasia" is preferable to "regeneration nodule."

* E. Blum, M.D. and M. Bevilacqua, Board-certified Pathologists, Department of Pathology, Variety Children's Hospital, Miami, Florida; W.A.D. Anderson, M.D., Professor of Pathology, University of Miami, School of Medicine, Miami, Florida; and M. D. Reuber, M.D., Associate Professor, Department of Pathology, University of Maryland, School of Medicine, and pathological consultant to the U.S. Environmental Protection Agency.

** World Health Organization, International Agency for Research on Cancer, Liver Cancer, Proceedings of a Working Conference, Lyon, 1972; Brit. Med. J., (5795), 261-262, 1972.

Table 5. The Tumorigenicity of Dieldrin in the Swiss-Webster Mouse
Examination of Liver Slides

Code No.	I.D. No. of Mouse	Sex	Diet Fed	Age at Death (mos)	Diagnosis by W.E. MacDonald, Ph.D. U. of Miami (1971)	Diagnosis by W.A.D. Anderson, M.D. U. of Miami (Sept. 1971)	Diagnosis by Drs. Blum & Bevilacqua Miami, Fla. (1971)*	Diagnosis by W.A.D. Anderson, M.D. U. of Miami (Sept. 1973)	Diagnosis by M.D. Reuber, M.D. Representing EPA (Mar. 1973)
1.	2835	M	Control	10	Leukemic infiltration	No neoplasia	Negative	No carcinoma	No carcinoma
5.	2856	M	"	21	Negative	No neoplasia	Minute inflammatory infiltrates	No carcinoma	No carcinoma
6.	4236	M	"	11	Hemangioma & duct proliferation	Hemangioma	Area of old infarction; focal chronic inflammation; cavernous hemangiomas	Hemangioma	Hemangioendothelioma
22.	2849	M	"	14	Amyloid		Minute inflammatory infiltrates; marked dilatation & protein precipitate; fibrosis.	Amyloid	Amyloid
11.	2955	F	"	16	Hepatoma 4+	Hepatoma	Large area of cystic dilatation of sinusoids & veins with hemorrhage; small area of atrophy.	Hepatoma, questionable carcinoma	Hepatocellular carcinoma
15.	2928	F	"	18	Hepatoma with fibrous component 4+	Not fibrosarcoma	Hepatoma with fibrous component.	Malignant tumor, type uncertain	Kupffer cell sarcoma
17.	2908	F	"	9	Duct adenoma		Negative	No carcinoma	No carcinoma
24.	2916	F	"	14	Question of neoplasia; question of malignancy.		Negative	Nodular hyperplasia	Hepatocellular carcinoma
3.	2998	M	D-3ppm	11	Slight hypertrophy & cloudy swelling	No neoplasia	Negative	No carcinoma	No carcinoma
7.	2987	M	"	8	Amyloid	No neoplasia, amyloid	Amyloid	Amyloid	Amyloid
13.	3006	M	"	15	Some hypertrophy	No neoplasia	Diffuse inflammation	Nodular hyperplasia, inflammation	Hepatocellular carcinoma
20.	4297	M	"	14	Amyloid		Focal atrophic with amyloid	Amyloid	Amyloid

* Most of the diagnoses by Drs. Blum and Bevilacqua were added to this table August 27, 1974.

The Tumorigenicity of Dieldrin in the Swiss-Webster Mouse
Examination of Liver Slides
(continued)

2.

Code No.	I.D. No. of Mouse	Sex	Diet Fed	Age at Death (mo)	Diagnosis by W.E. MacDonald, Ph.D. U. of Miami (1971)	Diagnosis by W.A.D. Anderson, M.D. U. of Miami (Sept. 1971)	Diagnosis by Drs. Blum & Bevilacqua Miami, Fla. (1971)*	Diagnosis by W.A.D. Anderson, M.D. U. of Miami (Sept. 1973)	Diagnosis by M.D. Reuber, M.D. Representing EPA (Mar. 1973)
9.	3102	F	D-3ppm	27	Hypertrophy, hyperplasia, nodulation	No neoplasia	Focal chronic inflammation	Nodular hyperplasia	Hepatocellular carcinoma
12.	2991	M	"	9	Some cloudy swelling	No neoplasia	Nuclei swollen, minute infiltrates	Degeneration	No carcinoma
16.	3071	F	"	16	Some cloudy swelling	No neoplasia	Small focal infiltrates	No carcinoma	No carcinoma
4.	3214	M	D-10ppm	13	Hypertrophy, hyperplasia, nodulation	No neoplasia	Congestion, early atrophy, focal infiltrates	Nodularity, degeneration, questionable hepatoma	Hepatocellular carcinoma
19.	3224	M	"	23	Hypertrophy, hyperplasia, nodulation		Atrophic changes with hyperplasia	Nodular hyperplasia, questionable hepatoma	Hepatocellular carcinoma
23.	3174	M	"	16	Hypertrophy, hyperplasia, nodulation		Small focal cellular infiltrates	Hepatoma, questionable hepatocarcinoma	Hepatocellular carcinoma
2.	3293	F	"	23	Hypertrophy, hyperplasia, nodulation	No neoplasia	Focal infiltrates, one area with ducts & marked inflammation	No carcinoma	No carcinoma
8.	3300	F	"	26	Hypertrophy, hyperplasia, nodulation, & leukemic infiltration		Focal infiltrates, cellular swelling	Neoplasia, probable carcinoma	Hepatocellular carcinoma
10.	3230	F	"	15	Hypertrophy, hyperplasia, nodulation	No neoplasia	Extensive necrosis, probably an infarct	Nodular hyperplasia, questionable hepatoma	Hepatocellular carcinoma
14.	3270	F	"	25	Hypertrophy, hyperplasia, nodulation	No neoplasia	Liver cells swollen	Hepatoma, questionable carcinoma	Hepatocellular carcinoma
18.	3285	F	"	14	Hydropic degeneration, duct proliferation		Severe hydropic degeneration	Degeneration, no carcinoma	Amyloid; focal large bile duct proliferation
21.	3287	F	"	18	Hypertrophy, nodulation, and duct adenoma		Bile duct adenomata	No carcinoma	No carcinoma; bile duct hyperplasia
25.	3254	F	"	23	Hypertrophy, nodulation & secondary sarcoma		Marked atrophic changes with large areas of fibrosis (cirrhosis)	Hepatocarcinoma	Hepatocellular carcinoma

*Most of the diagnoses of Drs. Blum and Bevilacqua were added to this table August 27, 1974.

Dr. M. D. Reuber recognized that there does exist a "gray area" in the diagnosis of pathological lesions in carcinogenic testing, and states that ... "The area that causes the most problems are the lesions in which there are focal atypical or malignant cells," and he asks ... "Should this be considered as a malignant tumor?" * In spite of these reservations, Dr. Reuber, in contrast to the diagnoses of the four other pathologists involved in this investigation, most frequently diagnosed a hepatic lesion as malignant.

The dilemma of the diagnosis of the pathological liver lesion remains unsolved, yet far-reaching decisions are being made by the Environmental Protection Agency and other agencies and laboratories regarding the carcinogenicity or noncarcinogenicity of a compound.

Wm. B. Henderson

* Report: Discussion Group #8, Criteria for Tumor Diagnosis and Classification of Malignancy, M. D. Reuber, Leader. Conference on Carcinogenesis Testing in the Development of New Drugs, National Academy of Sciences, Washington, D.C., May 23-25, 1973.

Part 2

EFFECTS OF FEEDING ALDRIN AND DIELDRIN ON THE TUMOR INCIDENCE IN ALBINO RATS

INTRODUCTION

This report presents results with two rat feeding experiments.

I. Feeding Experiment No. 1: Reference is made to the report published in 1970 by Deichmann et al.* which dealt with the feeding of aldrin and dieldrin to weanling Osborne-Mendel rats in concentrations of 0, 20, 30, and 50 ppm. In this publication it was stated that the overall tumor incidence (benign and malignant) in both male and female rats fed aldrin or dieldrin was significantly lower than the incidence of these tumors in male and female control rats. The paper concluded that "when fed for a lifetime to albino rats, aldrin and dieldrin (and endrin) are neither tumorigenic nor carcinogenic."

In the 1970 report, tumor deaths were related to the total number of rats with which the feeding experiment was initiated. (Consideration was not given to the reduced periods of survival of some of the groups of rats.) We now realize that it would have been more realistic to relate tumor incidence to the number of male and female rats that were actually at tumor risk. This eliminates those rats that, for various reasons, died during the early months of the experiment, namely, a total of six males and 46 females from a total number of 800 rats, or about 6 per cent. Thus, we considered that the number of rats at tumor risk were those that were alive at the beginning of the 11th month. The one tumor death before the 11th month, a female rat fed dieldrin 50 ppm, was included in the analysis.

In order to conduct the age adjusted statistical analysis of the tumor incidence, the statistician (Dr. P. F. Hunt, Statistics Unit, Sittingbourne Research Centre, England) was supplied with the following information for each animal:

- a) its period of survival,
- b) its cause of death, and
- c) tumors found or not found in the liver, mammary tissues, lymphoreticular system, uterus, ovaries, and other organs and tissues, as based on gross observation and microscopic examination and on the diagnosis of a tumor as either benign or malignant.

Tables 1 and 2 summarize these data. Dr. P. F. Hunt's report is as follows:

* Tumorigenicity of Aldrin, Dieldrin and Endrin in the Albino Rat. Ind. Med. and Surg., 39 (10), 426-434, 1970.

Table 1. SUMMARY - MALE RATS
TUMORIGENICITY OF ALDRIN AND DIELDRIN IN MALE RATS

This summary pertains ONLY to those rats that were alive at 11 months and that lived up to 31 months.

Group	No. of Rats Alive at 11 Mos.	Maximum Survival after Start of Feeding Experiment* (months)	Mean Survival after Start of Feeding Experiment* (months)	Rats with Tumors Dying Between:								11 Mos. until Termination	
				11 and 12 Mos.	13 and 18 Mos.	19 and 24 Mos.	25+ Months	Percent of Rats with Tumors		Percent of Rats with Tumors		Percent of Rats with Tumors	
				No. of Rats with Tumors	No. of Rats with Tumors	No. of Rats with Tumors	No. of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors
Control	100	28	20.6±4.8	0	6	6	8	6	6	8	8	20	20
Aldrin 20 ppm	50	28	19.4±4.2	0	2	4	1	2	4	1	2	5	10
Aldrin 30 ppm	50	29	19.7±4.1	0	2	4	2	3	6	2	4	7	14
Aldrin 50 ppm	50	31	20.2±3.7	0	2	4	1	1	2	1	2	4	8
Dieldrin 20 ppm	50	29	19.6±5.0	1	2	4	0	2	4	0	0	5	10
Dieldrin 30 ppm	46	29	21.0±4.1	0	1	2	2	4	9	2	4	7	15
Dieldrin 50 ppm	48	29	19.5±4.1	0	1	2	0	0	0	0	0	1	2

* This includes all rats whether or not they developed tumors.

Table 2. SUMMARY - FEMALE RATS
TUMORIGENICITY OF ALDRIN AND DIELDRIN IN FEMALE RATS

This summary pertains ONLY to those rats that were alive at 11 months and that lived up to 31 months.

Group	No. of Rats Alive at 11 Mos.	Maximum Survival after Start of Feeding Experiment* (months)	Mean Survival after Start of Feeding Experiment* (months)	Rats with Tumors Dying Between:										11 Mos. until Termination	
				11 and 12 Mos.		13 and 18 Mos.		19 and 24 Mos.		25+ Months					
				No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors	No. of Rats with Tumors	Percent of Rats with Tumors
Control	100	28	20.5±4.1	0	0	16	16	31	31	13	13	60	60	60	
Aldrin 20 ppm	49	26	18.9±3.9	2	4	5	10	10	20	3	6	20	41	41	
Aldrin 30 ppm	45	27	19.9±3.7	1	2	4	9	17	38	2	4	24	53	53	
Aldrin 50 ppm	28	25	18.5±3.8	1	4	4	14	6	21	0	0	11	39	39	
Dieldrin 20 ppm	50	29	20.5±3.1	2	4	4	8	15	30	3	6	24	48	48	
Dieldrin 30 ppm	41	29	19.2±4.3	2	5	2	5	11	25	1	2	16	39	39	
Dieldrin 50 ppm	39	28	19.7±4.8	1	3	1	3	12	31	2	5	16	41	41	

* This includes all rats whether or not they developed tumors.

Statistical Analysis of Tumour Incidence in the Deichmann
Aldrin/Dieldrin Rat Study No.1

(by Dr. P. F. Hunt)

A summary of the tumour incidence from this study is shown in Tables 3 and 4 for male and female rats respectively.

If no account is taken of the survival rates for the different treatment groups the tumour incidence can be considered solely in relation to the initial group sizes. Thus in this study where controls contained 100 rats and the other groups generally contained 50 rats and under the assumption that each rat, regardless of group, has the same chance to display a tumour, twice as many tumors would be expected in control as in each treatment group.

As an example of this consider the male rats with at least one tumour:-

		<u>C</u>	<u>A20</u>	<u>A30</u>	<u>A50</u>	<u>D20</u>	<u>D30</u>	<u>D50</u>	<u>Total</u>
Group Size	:	100	50	50	50	51	50	50	401
No. of animals with tumour (O)	:	20	3	8	3	5	6	1	46
Expected No. of tumours based on group size (E)	:	11.5	5.7	5.7	5.7	5.9	5.7	5.7	
O/E	:	1.74	0.53	1.40	0.53	0.85	1.05	0.18	

A χ^2 test would reject at the 5% level the assumption of equal tumour incidence among the groups. However, this type of analysis makes no allowance for the different survival times experienced by each treatment group.

Regardless of treatment an older animal is more likely to have a tumour than a younger animal. In consequence if the animals in one group have survived longer than another similar group they are likely to display more tumours.

The method of overcoming this problem is to analyse the study month by month so that within any period the tumour risk can be considered constant and the risk to each group will be proportional to the number of survivors in those groups at the start of the period. The expectations for each month are summed to produce the total number of expected tumours for each group. In the case of our example the figures are :-

DEICHMAN ALDRIN/DIELDRIN STUDY : TABLE 3 SUMMARY OF TUMOUR INCIDENCE IN MALE RATS

ANIMALS IN EACH TREATMENT		NUMBER OF ANIMALS WITH TUMOURS OF SPECIFIC TYPE												NUMBER OF ANIMALS WITH						
		LIVER			MAMMARY			LYMPHO- RETICULAR		PROSTATE		TESTES			OTHER		AT LEAST ONE TUMOUR	MORE THAN ONE TUMOUR	AT LEAST ONE MALIGNANT TUMOUR	MORE THAN ONE MALIGNANT TUMOUR
		B	M	N	B	M	N	B	M	B	M	B	M	N	B	M				
CONTROL	100	1	1	5	1			9			0	0	1	0	2	4	20	4	14	1
ALDRIN 20PPM	50	0	0	0	0			3			0	0	0	0	0	0	3	0	3	0
ALDRIN 30PPM	50	0	0	1	0			7			0	0	0	0	1	0	8	1	7	0
ALDRIN 50PPM	50	0	0	0	0			2			0	0	0	0	1	0	3	0	2	0
DIELDRIN 20PPM	51	0	0	1	0			3			0	0	0	0	1	0	5	0	3	0
DIELDRIN 30PPM	50	0	0	1	0			5			0	0	0	0	0	0	6	0	5	0
DIELDRIN 50PPM	50	0	0	0	0			0			0	0	0	0	1	0	1	0	0	0

Reproduced from
best available copy.

DEICEMAN ALDRIN/DIELDRIN STUDY : TABLE 4 SUMMARY OF TUMOUR INCIDENCE IN FEMALE RATS

TREATMENT	ANIMALS IN EACH TREATMENT	NUMBER OF ANIMALS WITH TUMOURS OF SPECIFIC TYPE										NUMBER OF ANIMALS WITH				
		LIVER		MAMMARY		LYMPHO- RETICULAR	UTERUS		OVARY		OTHER		AT LEAST ONE TUMOUR	MORE THAN ONE TUMOUR	AT LEAST ONE MALIGNANT TUMOUR	MORE THAN ONE MALIGNANT TUMOUR
		B	N	B	M		B	M	B	M	B	M				
CONTROL	100	0	0	24	9	10	2	3	0	0	2	1	44	7	20	3
ALDRIN 20PPM	49	0	0	8	2	1	4	0	1	0	0	1	16	1	4	0
ALDRIN 30PPM	50	0	0	17	3	1	5	0	0	0	0	0	23	3	4	0
ALDRIN 50PPM	50	0	0	6	2	2	2	0	0	0	0	2	10	2	4	1
DIELDRIN 20PPM	50	0	0	16	2	2	0	1	0	0	0	0	21	0	5	0
DIELDRIN 30PPM	48	0	0	6	3	1	4	0	0	0	1	1	13	3	5	0
DIELDRIN 50PPM	50	0	0	9	1	2	3	0	0	1	0	0	15	1	4	0

B : Benign Tumour
M : Malignant Tumour

		<u>C</u>	<u>A20</u>	<u>A30</u>	<u>A50</u>	<u>D20</u>	<u>D30</u>	<u>D50</u>
Expected No. of tumours based on survival (E)	:	13.6	4.7	5.5	5.3	5.9	6.4	4.7
O/E	:	1.47	0.63	1.46	0.57	0.85	0.94	0.22

An alternative way of considering the effect of the differences in survival would be to take the expectations above and calculate "adjusted initial group size" assuming identical survivability. This would give

"Adjusted initial size"	:	118	41	48	46	51	56	41
----------------------------	---	-----	----	----	----	----	----	----

From either consideration it can be seen that the differences in actual tumour numbers have been affected by survivability and that adjustment in this respect has reduced the differences between observed and expected tumours.

The significance of the χ^2 is now below 10%.

The principle tumour categories from Tables 3 and 4 have been analyzed by the above method and the results are given in Tables 5 and 6.

Results

Males: None of the variates gave significant results. The largest discrepancies between observed and expected tumours, either expressed as a difference or a ratio, generally occurred in control.

Females: None of the variates gave significant results.

Conclusion

There were no significant differences in tumour incidence between the treatment groups in this study. (Ref: T357/3226)

Dr. Hunt's conclusions support the conclusions reported by Deichmann et al. (1970) that, when fed for a lifetime to weanling male and female Osborne-Mendel rats, aldrin and dieldrin, in dosages of 20, 30, and 50 ppm, are neither tumorigenic nor carcinogenic.

TABLE 5

DEIGMAN ALDRIN/DIELDRIN STUDY : OBSERVED AND EXPECTED TUMOURS IN MALE RATS

TREATMENT	NUMBER OF ANIMALS WITH									
	AT LEAST ONE TUMOUR			MORE THAN ONE TUMOUR			WITH AT LEAST ONE MALIGNANT TUMOUR			O/E
	O	E	O/E	O	E	O/E	O	E	O/E	
CONTROL	20	13.6	1.47	4	1.7	2.37	14	10.1	1.39	
ALDRIN 20PPM	3	4.7	0.63	0	0.6	0.00	3	3.8	0.80	
ALDRIN 30PPM	8	5.5	1.46	1	0.5	1.84	7	4.0	1.75	
ALDRIN 50PPM	3	5.3	0.57	0	0.6	0.00	2	4.0	0.50	
DIELDRIN 20PPM	5	5.9	0.85	0	0.6	0.00	3	4.2	0.72	
DIELDRIN 30PPM	6	6.4	0.94	0	0.6	0.00	5	4.5	1.11	
DIELDRIN 50PPM	1	4.7	0.22	0	0.4	0.00	0	3.5	0.00	
χ^2 +			9.3			6.5			9.1	

+ χ^2 is distributed as χ^2 with 6 degrees of freedom

O : Observed number of tumours

O/E : Ratio O to E

E : Expected number of tumours based on surviving treatment group sizes throughout study

TABLE 6

DEICHLORAN ALDRIN/DIELDRIN STUDY : OBSERVED AND EXPECTED TUMOURS IN FEMALE RATS

TREATMENT	NUMBER OF ANIMALS WITH A MAMMARY TUMOUR										NUMBER OF ANIMALS WITH														
	BENIGN					MALIGNANT					TOTAL					AT LEAST ONE TUMOUR MORE THAN ONE TUMOUR					AT LEAST ONE MALIGNANT TUMOUR				
	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	
CONTROL	24	28.1	0.85	9	7.8	1.15	33	35.9	0.92	44	46.6	0.94	7	5.8	1.22	20	15.2	1.32							
ALDRIN 20PPM	8	9.0	0.89	2	2.1	0.96	10	11.1	0.90	16	14.8	1.08	1	1.7	0.59	4	4.9	0.81							
ALDRIN 30PPM	17	10.5	1.62	3	2.7	1.13	20	13.1	1.52	23	17.3	1.33	3	2.1	1.45	4	5.7	0.71							
ALDRIN 50PPM	6	4.7	1.27	2	1.1	1.90	8	5.8	1.39	10	7.8	1.29	2	0.8	2.43	4	2.7	1.51							
DIELDRIN 20PPM	16	14.5	1.10	2	3.7	0.55	18	18.2	0.99	21	23.9	0.88	0	2.8	0.00	5	7.5	0.67							
DIELDRIN 30PPM	6	8.9	0.67	3	2.1	1.44	9	11.0	0.82	13	14.7	0.89	3	1.7	1.74	5	4.8	1.05							
DIELDRIN 50PPM	9	10.2	0.88	1	2.7	0.38	10	12.9	0.78	15	17.0	0.89	1	2.1	0.47	4	5.4	0.74							
χ^2			6.7			3.3			6.3			3.9			7.1			4.2							

 χ^2 is distributed as χ^2 with 6 degrees of freedom

O : Observed number of tumours

E : Expected number of tumours

O/E : Ratio of O to E

page 25

Reproduced from
best available copy.

II. Feeding Experiment No. 2: A second rat feeding experiment with aldrin* was started on May 20, 1971, with two strains of female rats: Osborne-Mendel and Sprague-Dawley. These studies were initiated with the approval and support of NIH (ES 00052-07-S). Financial support for the completion of the feeding portion of this experiment and initiation of the mammary tumor histology was provided by EPA (R 801470), supplemented by the Shell Chemical Company.

The primary reason for the use of female rats was that in our first aldrin-dieldrin feeding experiment (Feeding Experiment No. 1; Deichmann et al., 1970), the tumors that developed most frequently were found primarily in females, i.e., mammary tumors. The decision to use females in Feeding Experiment No. 2 was made because signs of acute toxicity (tremors and convulsions in the animals suffering marked loss of body weight) reduced life span, and a marked increase in the ratio of liver-to-body weight were all noted primarily in the female Osborne-Mendel rats of Feeding Experiment No. 1. Finally, it was decided to employ two strains of rats since the susceptibility to tumors varies in different strains of the same species.

Osborne-Mendel Rats:

Group A - 50 weanlings (age 28-30 days), fed the control diet.

Group B - 50 weanlings, fed the control diet supplemented with 20 ppm aldrin.

Group C - 50 weanlings, fed the control diet supplemented with 50 ppm aldrin.

Sprague-Dawley Rats:

Group D - 50 weanlings (age 28 days), fed the control diet.

Group E - 50 weanlings, fed the control diet supplemented with 20 ppm aldrin.

Group F - 50 weanlings, fed the control diet supplemented with 50 ppm aldrin.

The Osborne-Mendel strain rats, like the rats in Feeding Experiment No.1, were raised in our laboratory from a colony obtained several years ago from the Food and Drug Administration in Washington, D.C. The Sprague-Dawley rats were purchased from the Sprague-Dawley Farm in Madison, Wisconsin. The protocol and experimental details were essentially like those of Feeding Experiment No.1, in that the animals were housed two or three in a hanging wire cage in quarters maintained at $76 \pm 3^\circ$ F. They were observed daily and were weighed twice a month, more often when indicated, i.e., when weight increased rapidly because of the growth of a tumor or when body weight was lost rapidly due to advanced age. Appearance and growth, as well as regression of tumors, were observed by visual inspection and recorded. Palpation of a tumor was avoided.

The experimental diets, prepared once a week, were analyzed periodically by gas-liquid chromatography to assure the desired concentrations and homogeneous distribution of aldrin. Pesticide concentrations in the control diet were: aldrin <0.1 ppm, p,p'-DDT <0.3 ppm, p,p'-DDE <0.1 ppm, and p,p'-DDD <0.2 ppm. A more extensive analysis carried out on one sample of control diet revealed the following concentrations: dieldrin <0.0004 ppm, and DDT plus DDE <0.0005 ppm.

* Formerly referred to as "Effect of aldrin as an inhibitor in the production of mammary tumors in rats..." (EPA Grant No. R801470, 1973-74).

Moribund animals were anesthetized by diethyl ether and sacrificed by exsanguination. Those that were found to lose body weight were removed from the racks and placed in cages on a table to permit closer observation. Every effort was made to avoid loss of tissues resulting from the death of an animal during the night or over the weekend.

At autopsy, all mammary tumors were taken and measured; in addition, samples of mammary tissue of all rats, as well as sections of organs showing gross changes, were taken and preserved for microscopic examination. Sections were also taken and preserved of the liver, lung, kidney, spleen, pancreas, heart, adrenal, ovary, uterus, mesenteric and mediastinal lymph nodes, stomach, and intestines of all animals (in the event that it should become desirable to study them histologically).

Results

Body Weights: Figures 1 and 2 present the body weights of the two strains of rats. The mean body weights of the Osborne-Mendel rats fed aldrin 20 and 50 ppm reached a plateau (360 g) of approximately 30 g below that of the control rats. The Sprague-Dawley rats fed aldrin 50 ppm gained weight more rapidly than the controls and reached a level of approximately 350 g, while those fed aldrin 20 ppm levelled off when their weights reached 315-320 g. The control animals reached a mean body weight of approximately 280 g. Almost every Osborne-Mendel and Sprague-Dawley rat began to lose body weight during the last months of the feeding experiment.

The significance of the body weight changes noted is not clear. It is noteworthy, however, that this is not the first time that investigators have noted that certain strains of rats fed organochlorine pesticides gained more rapidly than controls. We (Deichmann, *et al.*, 1972) reported this for weanling Osborne-Mendel rats fed aldrin 7.5 ppm, DDT 50 ppm, or DDT 50 ppm plus aldrin 7.5 ppm.

Survival: Of the 50 animals initiated per group, all animals of both strains were alive at age 120 days except for one Sprague-Dawley rat fed the control diet. The feeding of aldrin at 20 and 50 ppm to both strains of rats was accompanied by a dose-related reduced period of survival: the mean period of survival of the Osborne-Mendel control rats was 689 days; for the experimental rats mean survival was 640 and 570 days, respectively. The same observations were made for the Sprague-Dawley rats, except that their period of survival was shorter than that of the Osborne-Mendel rats: the mean period of survival of the Sprague-Dawley controls was 567 days, and that of the experimentals 540 and 409 days, respectively. The primary cause of death of these animals was pneumonitis, usually associated with advanced age and/or tumors of the mammary and uterine tissues. (Table 7)

Table 7 also presents the incidence of all tumors noted at necropsy in these animals. In agreement with our aldrin-dieldrin Feeding Experiment No.1, the Osborne-Mendel rats again demonstrated a high incidence of mammary tumors. Tumors of the uterus and ovaries followed in frequency. A few animals in all groups showed tumors in the lymph nodes, lungs, kidneys, adrenals, and spleen. A single control or experimental rat showed a tumor in either the heart, intestine, bladder, bone, or fat. No tumors were found in the liver of the control or experimental rats.

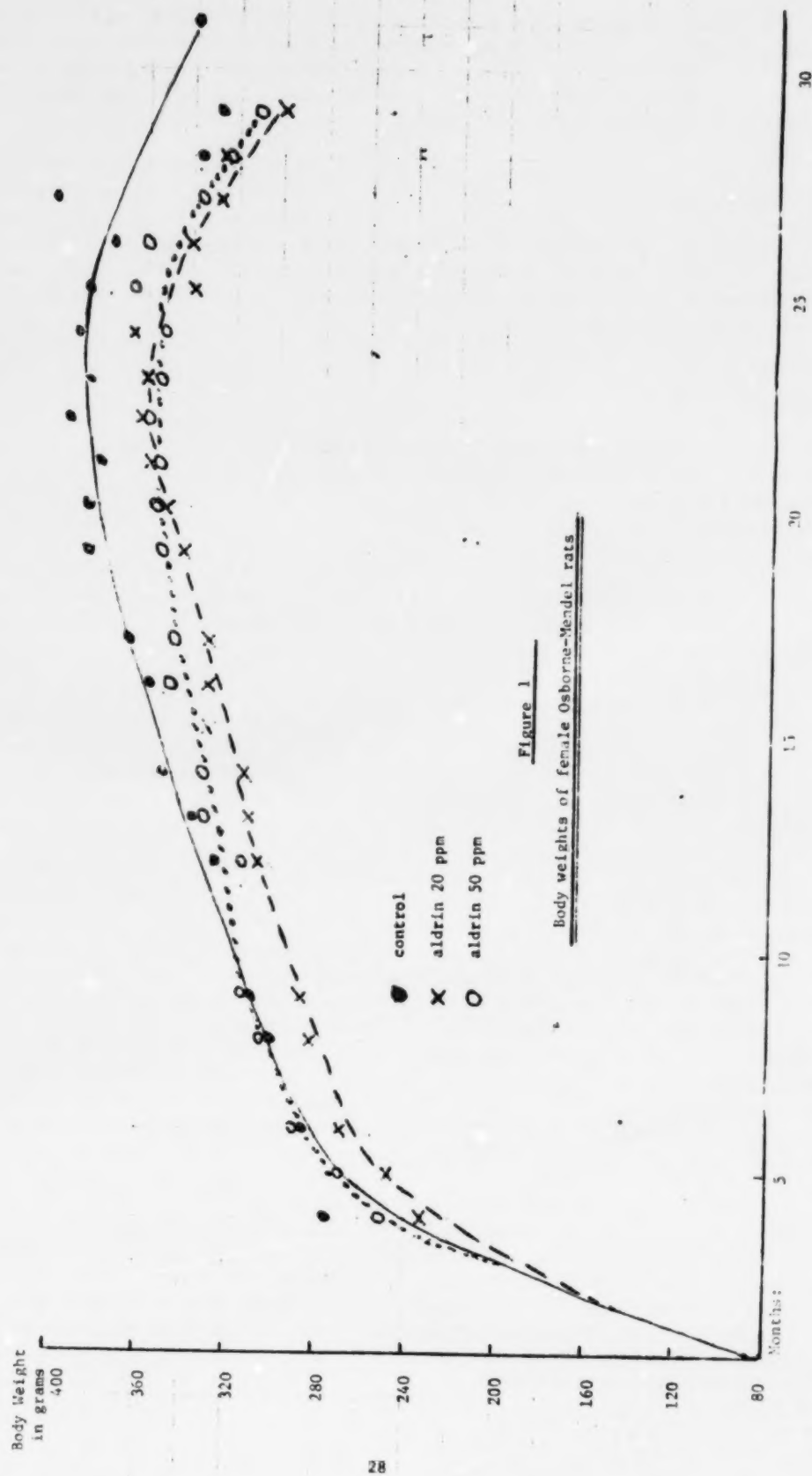


Figure 1
Body weights of female Osborne-Mendel rats

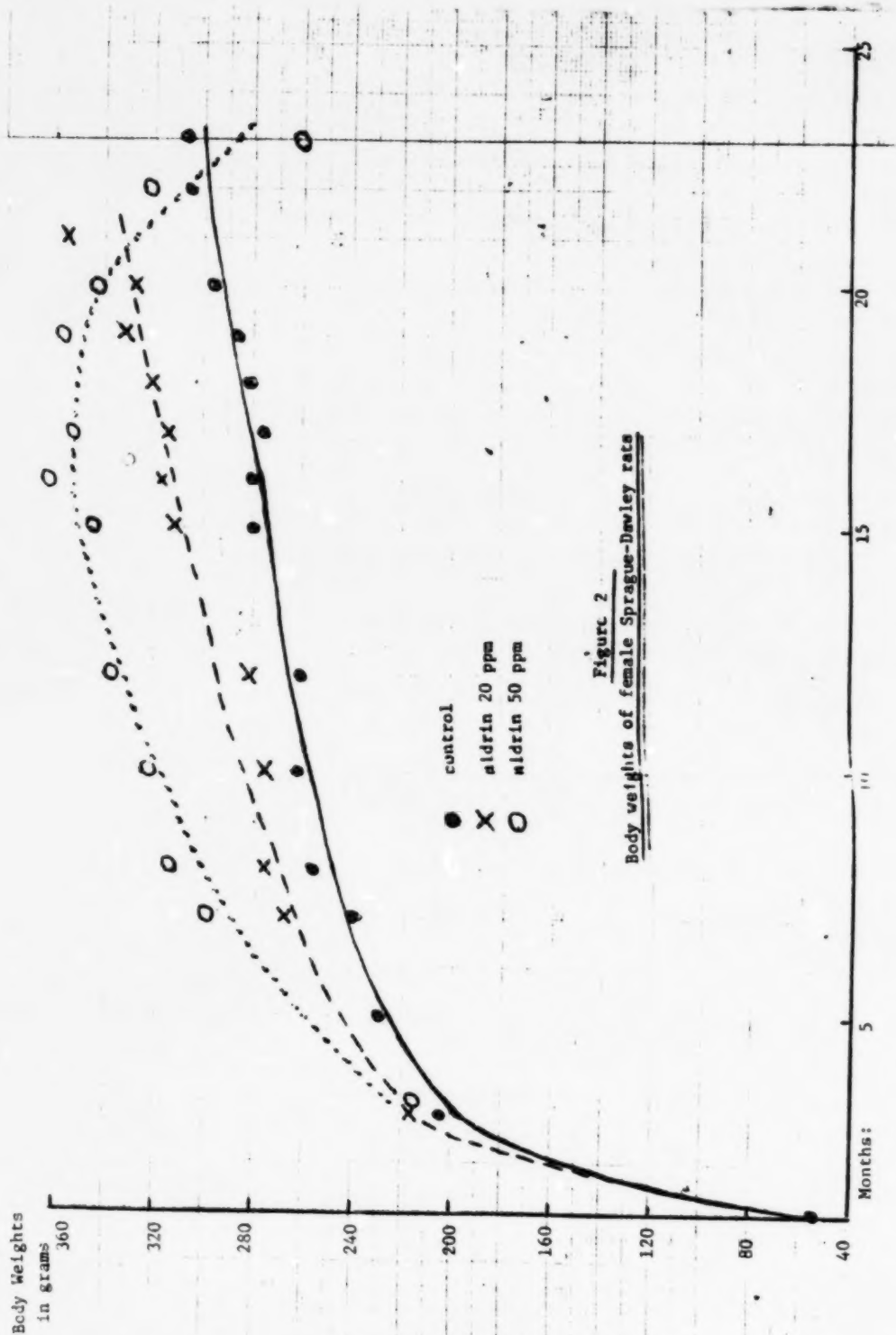


Figure 2
Body weights of female Sprague-Dawley rats

Table 7. INCIDENCE OF TUMORS (AT NECROPSY) IN RATS FED ALDRIN
(second aldrin rat feeding experiment)

Group	Diet Fed	No. of Rats at Age of 120 Days	Mean Survival of all Rats (days)	No. of Rats with Tumors	Mammary	Uterus	Ovaries	Lung	Liver	Lymph Nodes	Kidney	Adrenal	Heart	Intestine	Spleen	Bladder	Bone	Fat
A	Control	50	689	47	Osborne-Mendel Rats			3			7	3	1	1	1			1
					34	15	17											
					29	14	15											
B	Aldrin 20 ppm	50	640	46				5			12	2		1	6	1		
C	Aldrin 50 ppm	50	570	33	13	16	8	5			6	1	2				1	
D	Control	49	567	26	Sprague-Dawley Rats													
					5	18	5											
					3	15	8											
E	Aldrin 20 ppm	50	540	23						1			1					
F	Aldrin 50 ppm	50	409	18	2	14	2			1			1			1		

In the Sprague-Dawley rats (Table 7) the tumor incidence was lower than in the Osborne-Mendel rats. In this strain, uterine tumors were most frequent, followed by ovarian and mammary tumors. Occasionally, a single animal showed a tumor in the lymph nodes, adrenal, heart, or bladder. No tumors were found in the liver, kidneys, lungs, intestine, spleen, bone, or fat.

Since this feeding experiment was initiated largely for the purpose of studying the effect of aldrin on mammary tumors, tables were prepared for the statistician (Dr. P. F. Hunt), presenting the following information and data for a risk analysis of mammary tumor development:

- a) number of rats alive at the first of each month,
- b) number of rats showing first evidence of a mammary tumor during the month,
- c) number of deaths during each month,
- d) number of rats dying with mammary tumor(s) during the month,
- e) and number of rats "at risk" at the first of each month.

These data are summarized in Table 8 under the following headings:

- 1) number of rats per group,
- 2) number of rats with a single mammary tumor,
- 3) number of rats with multiple mammary tumors,
- 4) total number of rats with mammary tumors,
- 5) mean survival of all rats per group,
- 6) mean survival of rats that showed mammary tumors,
- 7) mean survival of the rats without mammary tumors,
- 8) mean age of the rats at the first appearance of a mammary tumor.

Osborne-Mendel Rats: The mammary tumor incidence was highest among control rats (34 versus 29 and 13 in the 20 and 50 ppm groups) (Table 8). The feeding of aldrin 20 ppm to Osborne-Mendel strain rats (when compared with the controls) did not markedly influence the mean latent period until the first tumor appeared (597 versus 590 days), nor did it markedly influence the mean survival of the rats (705 versus 696 days) that developed mammary tumors.

The Osborne-Mendel rats fed aldrin 50 ppm showed again (as in Feeding Experiment No.1) a marked reduction in survival. There was also a reduction in the mean latent period in the appearance of the first tumor.

Sprague-Dawley Rats: The most notable difference between the two strains of rats was the markedly lower number of Sprague-Dawley rats that developed single and/or multiple mammary tumors. While a total of 34 Osborne-Mendel control rats developed mammary tumors, only five Sprague-Dawley controls developed such tumors. The mean survival of the Sprague-Dawley control rats with tumors was 574 days, while the mean survival of the Osborne-Mendel controls was 705 days.

In the Sprague-Dawley rats fed aldrin 20 ppm, the mean age of an animal at the appearance of the first tumor was 668 days, while in the Osborne-Mendel rats fed aldrin 20 ppm, it was 590 days.

Table 8. EFFECT OF FEEDING ALDRIN ON MAMMARY TUMORS
IN FEMALE OSBORNE-MENDEL AND SPRAGUE-DAWLEY RATS
(second aldrin rat feeding experiment)

Group No.	Diet Fed	No. of Rats Started		No. of Rats with			Total No. of Rats with Tumors	Mean Survival of			Mean Age of Rat at Appearance of First Tumor (days)
		at Weaning (28 days)	at Age of 120 Days	Single Tumors	Multiple Tumors	All Rats (days)		Rats with Tumors (days)	Rats without Tumors (days)		
<u>Osborne - Mendel Rats</u>											
A	Negative Control Control Diet	50	50	21	13		34	689	705	655	597
B	Aldrin 20 ppm	50	50	23	6		29	640	696	563	590
C	Aldrin 50 ppm	50	50	9	4		13	570	671	534	537
<u>Sprague - Dawley Rats</u>											
D	Negative Control Control Diet	50	49	2	3		5	567	574	566	536
E	Aldrin 20 ppm	50	50	3	0		3	540	699	530	668
F	Aldrin 50 ppm	50	50	2	0		2	409	534	404	394

The mean survival of the Sprague-Dawley rats fed 50 ppm, when compared with those fed aldrin 20 ppm, was markedly reduced (Table 8).

Age Adjusted Risk Analyses: The risk analyses based on the data in Tables 7 and 8 were again conducted by Dr. P. F. Hunt*. These analyses (Table 9) were carried out in two ways: by considering deaths with a tumor in relation to rats alive at the start of each month, and by considering the number of rats first showing evidence of a tumor in relation to those which had shown no evidence of a tumor at the start of the month. One would expect that these two types of analysis would be in good agreement, which in fact was the case. The results in the two strains of rats were very similar.

The risk analysis studies showed that there were no significant differences between the respective controls and the aldrin-treated Osborne-Mendel and Sprague-Dawley rats when considered separately, although the lower survival in the aldrin 50 ppm Osborne-Mendel and Sprague-Dawley rats was a complication. The survival of the aldrin 20 ppm groups was only slightly less than the respective controls.

Analyses of the data presented showed no indication of an increase in mammary tumor incidence related to the feeding of 20 or 50 ppm of aldrin.

Summary

Based primarily on the incidence of all tumors (Table 7) found at necropsy in the female Osborne-Mendel and Sprague-Dawley rats fed aldrin in the diet at 0, 20, and 50 ppm, and on the age adjusted risk analyses (Tables 8 and 9) carried out on the number of rats that developed mammary tumors (predominantly in the Osborne-Mendel rats) -- it is concluded that the lifetime feeding of aldrin in concentrations of 20 and 50 ppm started at weaning age to female Osborne-Mendel and weanling female Sprague-Dawley rats neither increased nor decreased the incidence of tumors in these animals above that noted in the respective control groups.

NOTE: While this report with Osborne-Mendel and Sprague-Dawley female rats deals primarily with the number of tumors and the number of rats that developed tumors, we await the completion of the microscopic studies to determine whether the feeding of aldrin altered the distribution of benign and malignant tumors.

* Statistics Unit, Sittingbourne Research Centre, England.

Table 9. OBSERVED AND EXPECTED NUMBERS OF MAMMARY
TUMORS IN ANIMALS RECEIVING 20 OR 50 PPM ALDRIN

<u>Analysis of death with tumor</u>				<u>Analysis of first evidence</u>	
O		E	O		E
O/E			O/E		
<u>Osborne-Mendel Rats</u>					
Control	:	34	36	34	33.1
		0.95		1.03	
20 ppm	:	33	27.8	33	27.1
		1.19		1.22	
50 ppm	:	15	18.3	15	21.9
		1.03		0.69	
χ^2 (2df)	:	2.0		4.1	
<u>Sprague-Dawley Rats</u>					
Control	:	5	5.3	5	4.5
		0.94		1.11	
20 ppm	:	3	3.7	3	4.0
		0.82		0.75	
50 ppm	:	2	1.0	2	1.5
		1.94		1.36	
χ^2 (2 df)	:	1.1		0.5	

This investigation was conducted primarily by:

William B. Deichmann, PhD, MD(hon)., Professor of Pharmacology
and Director, Research and Teaching Center of Toxicology -
Principal Investigator.

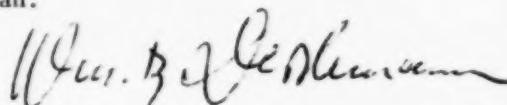
William E. MacDonald, Ph.D., Assistant Professor of Pharmacology,
and Associate Director, Research and Teaching Center of
Toxicology - Co-Principal Investigator.

Dr. P. F. Hunt, Statistician, Statistics Unit, Sittingbourne
Research Centre, England.

Dewey A. Cubit, B.S., Research Associate.

A. Gerald Beasley, M.S., Research Associate.

Mary A. Balkus, Laboratory Technician.

A handwritten signature in cursive script, appearing to read "W. B. Deichmann", is written in dark ink.

REFERENCES

Deichmann, Wm. B., MacDonald, W.E., Blum, E., Bevilacqua, M., Radomski, J., Keplinger, M., and Balkus, M. Tumorigenicity of Aldrin, Dieldrin, and Endrin in the Albino Rat. *Ind. Med. Surg.*, 39: 426-434, 1970.

Hunt, P. F. (Statistics Unit, Sittingbourne Research Centre, Sittingbourne, Kent, England). Personal communication, 1974.

Deichmann, Wm.B., MacDonald, W.E., Cubit, D.A., and Beasley, A.G. Effects of Starvation in Rats with Elevated DDT and Dieldrin Tissue Levels. *Int. Arch. Occ. Hlth* (*Int. Arch. Arbeitsmed.*), 29: 1-20, 1972; *Pesticides and the Environment: A Continuing Controversy* (published by Intercontinental Medical Book Corp., New York, 1973), p. 11-12.

Part 3

Mouse Reproduction Study

Seven-Generation Study: Retention of Dieldrin and DDT in the Tissues of Mice Fed Aldrin and DDT

In 1970, M. L. Keplinger, Wm. B. Deichmann and F. Sala published a report on the "Effects of Combinations of Pesticides on Reproduction in Mice." Even though the concentrations of dieldrin and DDT in abdominal body fat were determined in these mice, the data were not included in the report. There was a definite indication that succeeding generations fed aldrin 3 and 5 ppm retained higher concentrations of this pesticide in body fat.* This observation, without confirmation, was considered alarming and it was largely for this reason that this part of the investigation was repeated. All mice of the 1970 six-generation study were kept frozen at -5°C for varying periods of time (1 to 15 months) prior to analysis for organochlorine pesticides. It was also considered, at that time, that increasing concentrations of dieldrin and DDT in abdominal fat do not necessarily reflect an increasing total body load of pesticide. In the investigation reported here, both the concentration and the total retention of pesticides were determined in abdominal fat and in the total carcass.

Experimental

Diets Fed: The parent (P) generations of Swiss-Webster mice were selected randomly from our laboratory stock at weaning age (3-4 weeks; 9-12 g body weight). The animals were divided into three experimental groups of 10 females and six males each and were fed ground Purina Laboratory Chow supplemented as follows:

Group 1 - aldrin** 5 ppm; Group 2 - aldrin 10 ppm; Group 3 - DDT*** 100 ppm. Group 4 - 20 females and 12 males, which served as controls, were fed the uncontaminated control diet for seven generations.

The P, F₁ to F₃ and F₅ to F₇ generations of Groups 1 to 3 were fed the respective experimental diets; all F₄ generations of Groups 1, 2, and 3 were fed the control diet from weaning to sacrifice.

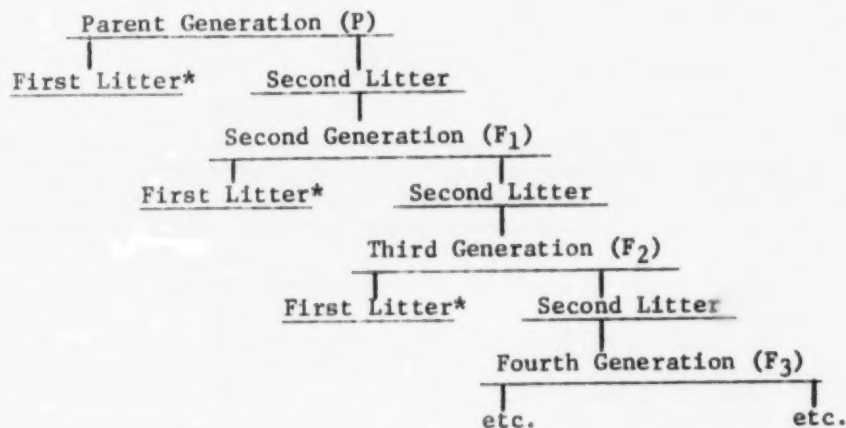
* Reported to NIH in October 1968 (NIH Grant ES00052-06).

** p,p-DDT 99+% [1,1-bis-(p-chlorophenyl)-2,2,2-trichloroethane], Aldrich Chemical Co., Inc.

*** Technical aldrin 95% (hexachlorohexahydro-endo, exo-dimethanonaphthalene), Shell Chemical Co.

The experimental diets were prepared in 50-pound batches (22.7 kg) with the pesticide dissolved in 100 ml of corn oil. The same volume of corn oil was added to the control diet of ground Purina Laboratory Chow. Each batch was analyzed to assure the correct pesticide content. In the control diet, the degree of contamination by pesticides remained below the following concentrations: aldrin: < 0.1 ppm; p,p'-DDT: < 0.3 ppm; p,p'-DDE: < 0.1 ppm; and p,p'-DDD: < 0.2 ppm. An extensive analysis carried out on one sample of the control diet revealed the following concentrations: aldrin and dieldrin < 0.0004 ppm; DDT plus DDE < 0.0005 ppm.

Breeding Schedule: The males were placed with the females when the P generation mice reached the age of 120-130 days. The first litters of the P and of each succeeding generation were discarded when they reached weaning age (21 days). After a 7 to 9 day rest period, the females were again placed with the males. (Usually three females were placed with one male in the breeding box.) If conception did not occur within two weeks, the male was replaced by one that had demonstrated virility. As the generations progressed from F₃ to F₆, conception was frequently delayed for three to four weeks. A few female mice of the F₅ and F₆ generations that had not conceived within one month were eliminated from the breeding schedule. The breeding procedure was maintained for seven generations as follows:



(* Discarded)

When the second litters reached weaning age, the parents (age 260± 15 days) were sacrificed (over a period of two weeks) and the analysis of their tissues was initiated immediately.

Analysis of Tissues for Dieldrin, p,p'-DDT, p,p'-DDE, and p,p'-DDD:
The animals were sacrificed by cervical dislocation, weighed, depilated with a commercial thioglycolate preparation, then washed with water and dried with absorbant paper. After a portion (approx. 0.2 g) of abdominal fat was removed from each mouse, the whole animal was minced with scissors, then macerated in a cooled 200 ml Sorvell Omnimixer with an equal volume of 0.73% saline until a slurry of uniform consistency was obtained. Total lipids and their pesticide contents were extracted (by the method of Folch *et al.*, 1957) from a 2.0 g aliquot of the slurry, representing 1.0 g of mouse. Pesticide residues were determined in the total lipid fraction and in samples of abdominal fat without additional further clean-up (by the method of Radomski and Fiserova-Bergerova, 1965). The pesticide residues were analyzed by gas-liquid chromatography, using Micro Tek 2000 R gas-chromatographs equipped with tritium foil electron capture detectors. Columns were of boro-silicate glass 6' x 1/4" OD packed with 10% DC 200/5% QF-1 coated on chromosorb W (HP) and 1.50% OV-17/1.95% QF-1 coated on chromosorb W (HP) and 1.50% OV-17/1.95% QF 1 on chromosorb W (HP).

Results

Body Weights: The mean body weights of the control mice and the experimental mice fed either aldrin 5 or 10 ppm, or DDT 100 ppm, at sacrifice (age 260±15 days), are presented in Table 1. While there were some statistically significant differences ($p < 0.05$) in the body weights of some of the groups of these animals over seven generations, there was no significant pattern.

Total Lipids: Comparison of the F_1 with the P generations of all groups of mice showed an increase in total body lipids (g/mouse). The increases were statistically significant ($p < 0.05$) in all groups except in the female control mice. The increase in total lipids was dramatic in male mice fed aldrin 10 ppm. In subsequent generations, total body lipids showed no specific pattern except in the male and female mice fed aldrin 10 ppm, in which total body lipids remained consistently elevated from the F_1 to F_6 generations. (Table 2)

Concentrations of Pesticides in the Lipids of the Total Carcass and Abdominal Fat: Since organochlorine pesticides may not be distributed uniformly in all fatty tissues of the body, concentrations of dieldrin, p,p'-DDT, p,p'-DDE, and p,p'-DDD were determined in the total lipids of the entire carcass and in abdominal fat taken at necropsy (Table 3 and Figures 1-3). The pesticide concentrations in total body lipids and in abdominal fat were essentially the same. Statistically significant differences were noted only in males and females of the P generation mice fed DDT 100 ppm. The mean concentrations of total DDT in the abdominal fat of male and female mice fed DDT 100 ppm were 980 and 950 ppm, respectively (Figure 3), while in the lipids of the entire carcass, the mean concentrations of total DDT were 514 and 423 ppm, respectively (Table 3).

Table 1

Body Weights of Mice (g) at Time of Sacrifice
Mean and Standard Deviation per Generation
 (Ages of Mice: 260 $\pm$ 15 Days)

Compounds Fed	Sex	G E N E R A T I O N S						
		P	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆
Control	M	35.3 $\pm$ 2.0 (4)*	42.2 $\pm$ 4.2 (11)	40.0 $\pm$ 4.2 (12)	38.9 $\pm$ 2.7 (11)	38.5 $\pm$ 3.4 (11)	32.2 $\pm$ 2.6 (11)	36.4 $\pm$ 1.8 (9)
	F	36.9 $\pm$ 3.3 (19)	36.8 $\pm$ 4.8 (19)	39.2 $\pm$ 4.8 (20)	35.2 $\pm$ 3.4 (18)	33.3 $\pm$ 3.0 (15)	36.8 $\pm$ 4.2 (17)	36.2 $\pm$ 2.2 (13)
Aldrin 5 ppm	M	40.0 $\pm$ 2.8 (5)	40.1 $\pm$ 3.3 (6)	40.3 $\pm$ 6.3 (6)	37.2 $\pm$ 7.1 (6)	37.7 $\pm$ 4.9 (6)	33.2 $\pm$ 2.5 (5)	42.2 $\pm$ 3.2 (6)
	F	34.0 $\pm$ 2.3 (10)	37.5 $\pm$ 6.4 (10)	35.8 $\pm$ 3.4 (9)	35.1 $\pm$ 3.0 (9)	35.7 $\pm$ 4.4 (7)	33.2 $\pm$ 4.9 (7)	35.2 $\pm$ 3.3 (6)
Aldrin 10 ppm	M	33.1 $\pm$ 2.7 (13)	46.4 $\pm$ 4.2 (6)	38.7 $\pm$ 1.8 (6)	39.5 $\pm$ 3.9 (5)	40.0 $\pm$ 5.7 (6)	39.4 $\pm$ 2.9 (6)	36.5 $\pm$ 2.8 (7)
	F	33.0 $\pm$ 3.5 (8)	38.2 $\pm$ 3.3 (8)	36.8 $\pm$ 3.4 (9)	36.4 $\pm$ 4.3 (9)	37.6 $\pm$ 5.6 (9)	38.6 $\pm$ 1.4 (6)	37.4 $\pm$ 4.0 (7)
DDT 100 ppm	M	34.6 $\pm$ 1.4 (4)	36.0 $\pm$ 2.3 (5)	38.9 $\pm$ 2.8 (5)	39.0 $\pm$ 3.8 (6)	33.4 $\pm$ 4.8 (6)	34.7 $\pm$ 4.2 (6)	41.7 $\pm$ 4.9 (5)
	F	32.2 $\pm$ 1.1 (9)	37.2 $\pm$ 3.7 (8)	35.7 $\pm$ 3.8 (10)	37.1 $\pm$ 6.9 (10)	30.4 $\pm$ 3.4 (8)	39.1 $\pm$ 3.4 (5)	41.0 $\pm$ 2.0 (3)

* Number of mice per group

Table 2

Total Lipids (g/mouse)
(Mean and Standard Deviation per Generation)

Compound Fed	Sex	G E N E R A T I O N S						
		P	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆
Control	M	2.92±0.46	6.31±1.32	5.60±1.14	6.38±1.63	4.57±1.14	2.50±1.09	6.85±2.70
	F	4.02±0.69	5.06±1.29	5.38±2.60	4.17±1.14	3.64±1.32	2.94±0.40	5.20±2.61
Aldrin 5 ppm	M	3.88±0.80	6.77±3.08	7.90±2.74	7.18±2.56	7.68±3.95	2.97±3.95	8.65±2.21
	F	3.64±1.02	6.32±3.04	6.13±2.82	6.30±1.23	7.02±5.79	2.18±0.48	6.86±2.24
Aldrin 10 ppm	M	2.12±0.53	10.90±1.63	6.60±1.71	8.85±1.35	7.26±3.56	5.14±1.21	6.18±2.52
	F	3.04±1.38	6.90±1.52	5.94±3.98	7.79±1.89	7.01±4.85	5.20±1.91	7.71±3.39
DDT 100 ppm	M	2.25±0.52	5.44±1.11	7.19±1.29	5.42±1.21	3.29±1.31	2.26±0.77	7.37±3.85
	F	2.86±1.12	6.28±2.08	5.61±1.57	6.63±2.10	2.60±0.59	3.87±0.59	5.90±1.19

Parent (P) to F₃ Generations Fed Dieldrin: In the second generation mice (F₁) fed aldrin 5 ppm, the mean concentration of dieldrin in the lipids of the entire carcass rose to a value of 50% above the concentration found in the P generation (mean values: 34-51 ppm), (Table 3). Total retention of dieldrin in the entire carcass of these mice rose approximately three times above the quantities found in the P generations (mean values: 0.12 to 0.37 mg/mouse), (Figure 4). No further significant changes in concentration and total retention of pesticides occurred in the F₂ and F₃ generations.

In the second generation (F₁) male and female mice, when compared to the P generations fed aldrin 10 ppm, the increases in mean dieldrin concentration in the lipids of the entire carcass approximately doubled (mean values: 60 to 118 ppm), (Table 3). Total retention increased almost eight-fold in the mice fed aldrin 10 ppm (mean values: 0.13 to 1.0 mg/mouse), (Figure 5). No further statistically significant changes in the concentration and total retention of dieldrin in male and female mice fed aldrin 10 ppm occurred in the F₂ and F₃ generations.

Parent (P) to F₃ Generations Fed DDT: In the mice fed DDT 100 ppm, the concentration of total DDT in the lipids of the entire carcass increased markedly (69%) from the P to the second (F₁) generation. Retention of total DDT in the total body also increased significantly, approximately five-fold in the second generations (mean values: 1.1 to 5.2 mg/mouse). Comparison of the concentration and retention of total DDT in the male and female mice of the F₂ and F₃ generations fed DDT 100 ppm showed no statistically significant changes when compared with the F₁ generation mice.

F₄ Generations: The question was raised whether the increases in pesticide concentration in the F₁, F₂, and F₃ generation mice were possibly related to pesticide-induced inherited characteristics. To test this hypothesis, all groups of the F₄ generation experimental mice were fed the control diet from weaning to sacrifice, with the feeding of pesticide diets resumed to the F₅ and F₆ generations.

At sacrifice of all experimental F₄ generation mice (which had absorbed pesticides in utero and via mother's milk, and ingested the pesticide-free diet from weaning to death), it was found that concentrations of dieldrin, p,p'-DDT, p,p'-DDE, and p,p'-DDD in the lipids of the total body and in abdominal fat, as well as the retention of these pesticides in the total carcass, were below the levels of sensitivity of the analytical method.

Briefly summarizing: in the Swiss-Webster mouse, the retention of dieldrin following the feeding of aldrin with the diet to four generations (P, F₁, F₂, and F₃) led to a significant increase in the concentration of dieldrin in the lipids of the total carcass and abdominal fat, and to a significantly increased total retention of dieldrin in the total carcass in the second generation (F₁). There were further gradual, though not statistically significant, increases in concentration in the total retention of dieldrin in F₂ and F₃ generation mice. The fifth (F₄)

Figure 1

Dieldrin (ppm) in Abdominal Fat of Mice Fed Aldrin 5 ppm

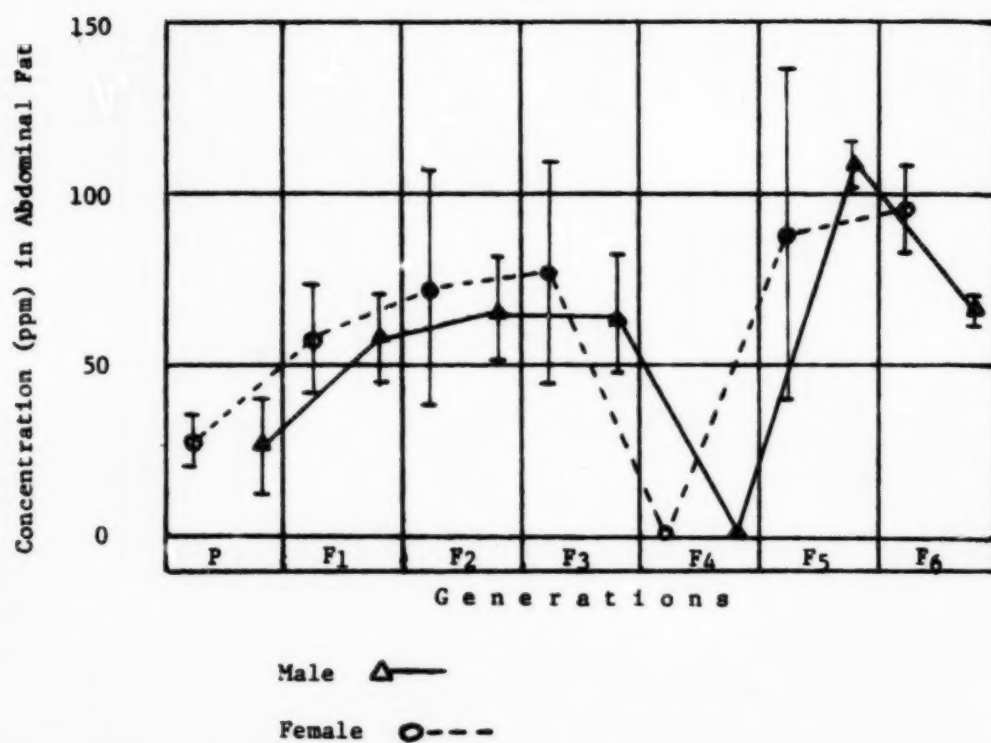


Figure 2

Dieldrin (ppm) in Abdominal Fat of Mice Fed Aldrin 10 ppm

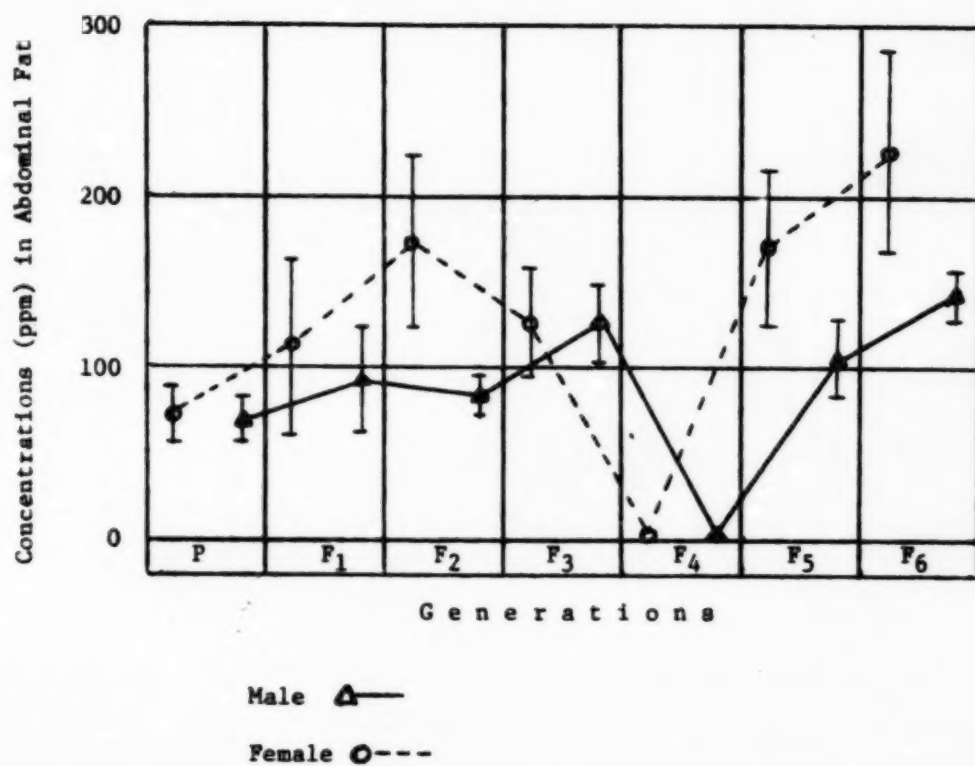


Figure 3

Total DDT (ppm) in Abdominal Fat of Mice Fed DDT 100 ppm

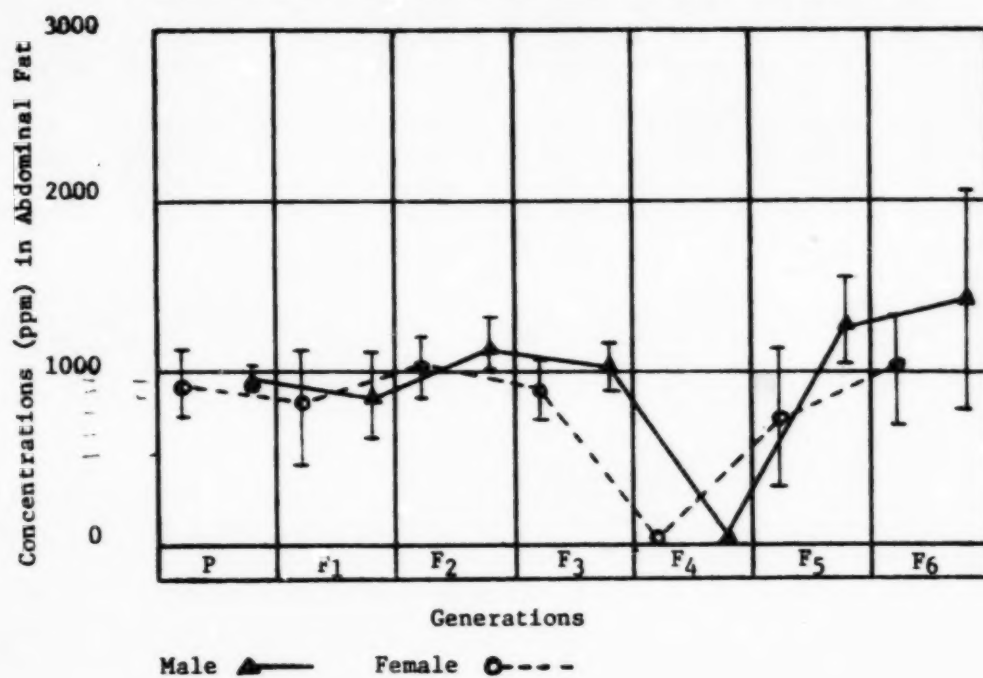


Table 3

Dieldrin and Total DDT Concentrations (ppm)
in Total Lipids of Entire Carcasses
(Mean and Standard Deviation per Generation)

Compound Fed	Sex	GENERATIONS							
		P	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	
Control	M	<	<	<	<	<	<	<	
	F	<	<	<	<	<	<	<	
Aldrin 5 ppm	M	31± 10	50± 7	56± 5	50± 17	<	88± 16	56± 7	
	F	38± 10	52± 7	66± 6	75± 35	<	93± 53	67± 75	
Aldrin 10 ppm	M	60± 12	91± 12	89± 8	119± 12	<	115± 5	121± 30	
	F	60± 16	145± 40	136± 8	114± 25	<	149± 64	159± 19	
DDT									
100 ppm	M	p,p'-DDT	468± 51	810±181	991±121	954±233	<	649±145	815± 26
		p,p'-DDE	35± 12	66± 10	38± 29	49± 36	<	29± 11	48± 31
		p,p'-DDD	11± 2	34± 6	10± 9	36± 13	<	25± 7	58± 42
		Total DDT	514± 63	910±193	1039±184	1039±275	<	703±163	921± 44
	F	p,p'-DDT	363± 49	585±127	754±130	865±179	<	602±154	573± 99
		p,p'-DDE	29± 9	48± 32	34± 32	26± 24	<	30± 16	73± 44
		p,p'-DDD	31± 15	31± 10	36± 20	42± 14	<	25± 15	51± 20
		Total DDT	423± 74	664±160	824±184	933±194	<	567±185	697±159

Minimum detectable levels: dieldrin <1.0 ppm
p,p'-DDT <2.0 ppm
p,p'-DDE <1.0 ppm
p,p'-DDD <1.0 ppm

Figure 4

Dieldrin in Total Carcass of Mice Fed Aldrin 5 ppm (mg/mouse)

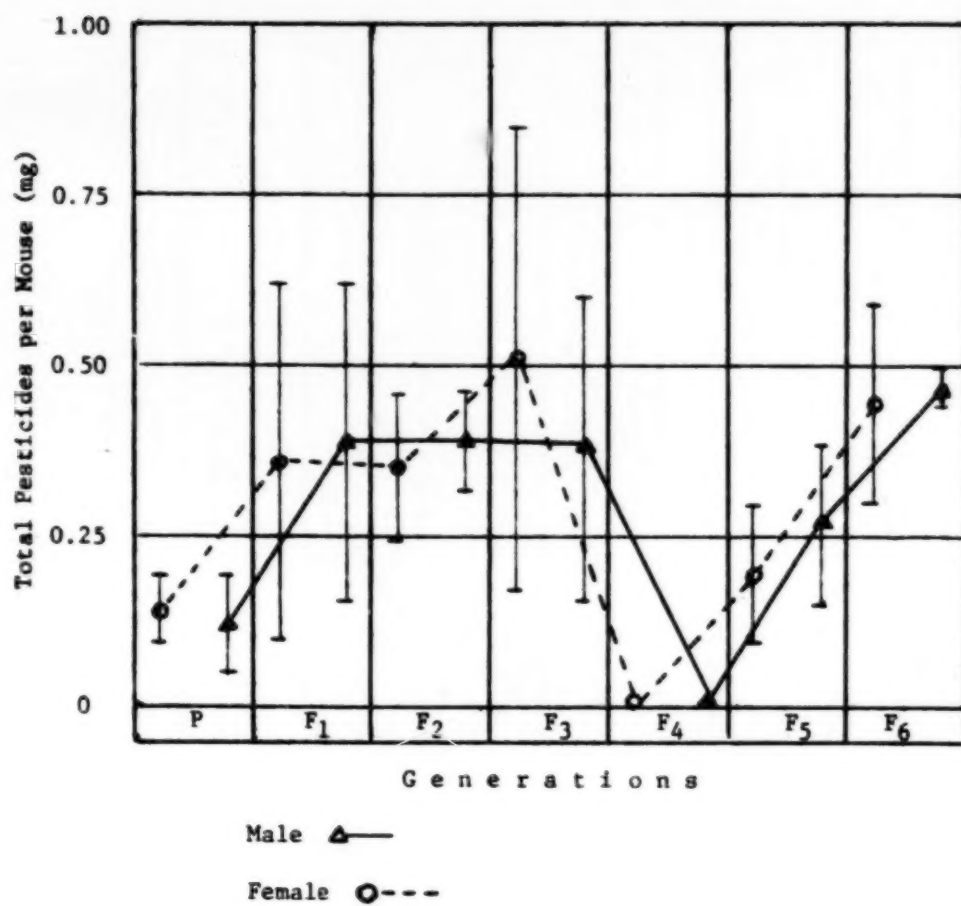


Figure 5

Dieldrin in Total Carcass of Mice Fed Aldrin 10 ppm (mg/mouse)

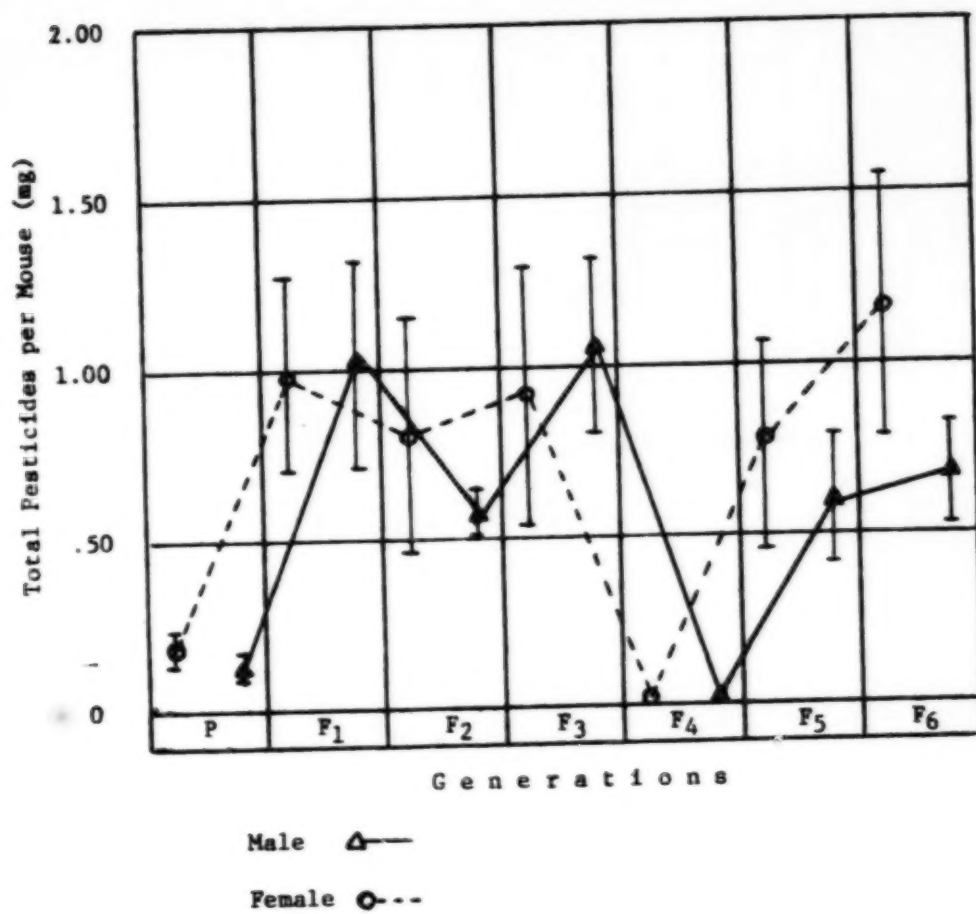
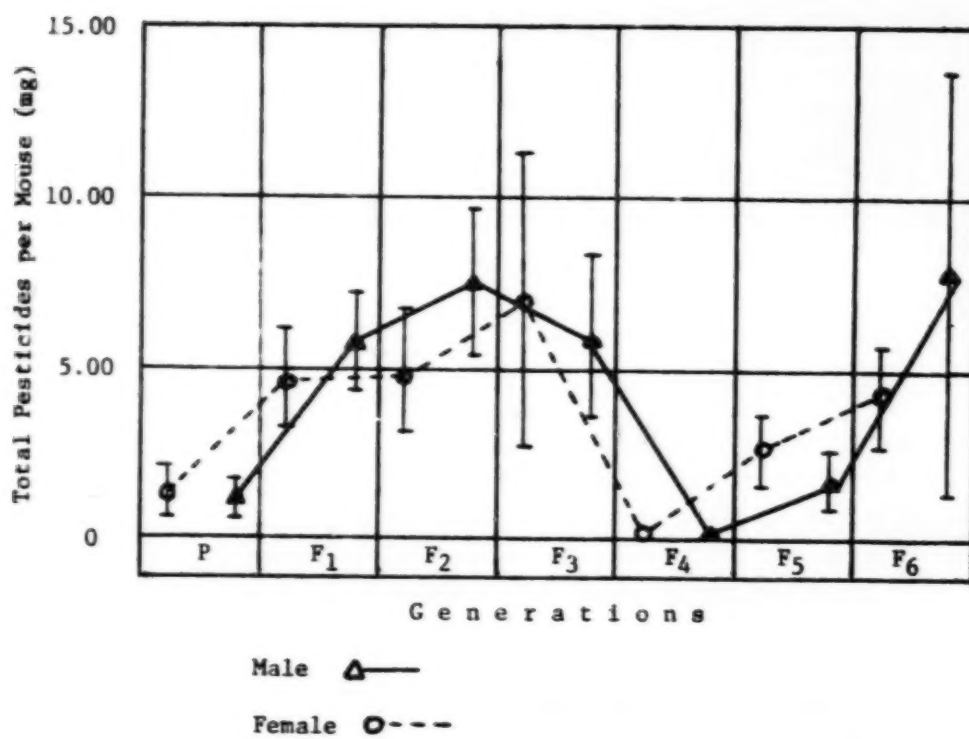


Figure 6

Total DDT in Total Carcass in Mice Fed DDT 100 ppm (mg/mouse)



generations of experimental mice, born of parents that carried a considerable load of dieldrin, were fed from weaning the control diet, leading to a complete depletion of this pesticide even though the animals had absorbed dieldrin in utero and via lactation. Concentration and retention of DDT followed the same general pattern as that of dieldrin.

Comparison of the Concentration and Total Body Retention of Pesticides in P and F₅ Generations: The P and F₅ generations provide "comparable" baseline data. They were essentially identical, differing only in that (1) the parents of the P generations, in contrast to the parents (F₄) of the F₅ generations, did not absorb pesticides in utero and in mothers' milk, and that (2) the parents and grandparents of the P generation mice had no (known) exposure to aldrin (dieldrin) or DDT and its metabolites, while the parents (namely, F₄) of the F₅ generations were the offspring of parents, grandparents, and great grandparents that had ingested these pesticides throughout their lifetime. Comparison of the pesticide concentration and total retention in P and F₅ generations reveals the following (for both sexes combined) mean values:

Aldrin 5 ppm

Mean concentration of dieldrin in the lipids of the entire carcass:

P generation	35 ppm
F ₅ generation	90 ppm

Mean retention of dieldrin in total carcass:

P generation	0.13 mg
F ₅ generation	0.23 mg

Aldrin 10 ppm

Mean concentration of dieldrin in the lipids of the entire carcass:

P generation	60 ppm
F ₅ generation	132 ppm

Mean retention of dieldrin in total carcass:

P generation	0.15 mg
F ₅ generation	0.69 mg

DDT 100 ppm

Mean concentration of total DDT in the lipids of the entire carcass:

P generation	468 ppm
F ₅ generation	635 ppm

Mean retention of total DDT in total carcass:

P generation	1.34 mg
F ₅ generation	2.13 mg

Conclusions

The concentration of dieldrin or p,p'-DDT and metabolites in the entire body and the total retention of these pesticides in the entire carcass were determined over seven generations of Swiss-Webster mice fed diets supplemented with aldrin 5 or 10 ppm or DDT 100 ppm.

As the generations progressed, particularly from F₃ on, conception was frequently delayed for three to four weeks. When pregnancy did not occur within one month, the male was replaced by one of proven fertility. Other signs of toxicity, such as tremors and reduction in body weight, were not observed.

When sacrificed at age 260 $\pm$ 15 days, total body lipids in the F₁ generations of all groups showed statistically significant increases when compared with the respective P generations. In subsequent generations, total body lipids showed no specific trend with the exception of the mice fed aldrin 10 ppm. In these animals, total body lipids remained elevated from F₁ to F₆ generations.

In all generations, the pesticide concentrations in abdominal fat were essentially the same as the concentrations in the total body lipids with one notable exception, namely, in the mice of the P generation fed DDT 100 ppm. The mean concentrations of DDT and its metabolites in the abdominal fat of these animals were approximately twice the concentrations in the lipids of the total body.

Mice fed aldrin 5 or 10 ppm, or DDT 100 ppm, showed a significant increase in both concentration and total retention of dieldrin or DDT and metabolites in the total carcass of the second generation (F₁) when compared with the concentration and retention of these compounds in the P generation mice. No further significant changes occurred in generations F₂ and F₃.

Feeding of the control (pesticide-free) diet to all groups of the F₄ generation experimental mice from weaning to sacrifice resulted in the complete excretion of pesticides absorbed by the animals while in utero and with their mothers' milk.

Resumption of the feeding of aldrin 5 and 10 ppm, and DDT 100 ppm, to generations F₅ and F₆ resulted, in the F₅ generation, in markedly elevated total body pesticide levels and in markedly increased concentrations of pesticides in the total body lipids (when compared with the concentration and retention in the P generation), and to further increases in concentration and total pesticide retention in the F₆ generation.

The increase, in both pesticide concentration and retention, in the F₅ generation above that of the P generation suggests that pesticide-induced inherited characteristics played a role in modifying the mechanism underlying pesticide retention. It will require further analyses of these data and additional studies to find the answer.

Primary contributions to this investigation were made by the following:

William B. Deichmann, Principal Investigator
William E. MacDonald, Co-Principal Investigator
Dewey A. Cubit, Chemist
A. Gerald Beasley, Chemist
Mary D. Balkus, Technician

W. B. Deichmann

References

- Keplinger, M.L., Deichmann, Wm.B., and Sala, F.
Effects of combinations of pesticides on reproduction in mice.
(Presented at the Sixth Inter-American Conference on Toxicology
and Occupational Medicine, August 1968); published in
Pesticides Symposia by Hales and Associates, Inc., Miami,
Florida, 1970 (p. 125-133).
- Radomski, J.L. and Fiserova-Bergerova, V.
The determination of pesticides in tissues with the electron
capture detector without prior clean-up.
Ind. Med. Surg., 34: 934-939, 1965.
- Deichmann, Wm. B.
Progress Report to National Institutes of Health (Grant No.
ES 00052-06), 1969.
- Folch, J., Lees, M., Sloan, S.G.H.
A simple method for the isolation and purification of total
lipids from animal tissues.
J. Biol. Chem., 226: 497-509, 1957.
- Deichmann, Wm.B., MacDonald, W.E., and Cubic, D.A.
DDT tissue retention: sudden rise induced by the addition of
aldrin to a fixed DDT intake.
Science, 172: 275-276, 1971.

Reproduced from
best available copy.